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16. ABSTRACT

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SENSITIVE BIOCHEMICAL AND BEHAVIORALINDICATORS OF TRACE SUBSTANCE EXPOSURE - PART 1, CERIUM

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FOREWORD

The many benefits of our modern, developing, industrial societh are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides of proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

With the advent of the automotive catalytic converter as a means of reducing mobile source emissions, research was undertaken to define the pharmacological and behavioral effects of the convertor attrition products, platinum and cerium. This data, in conjunction with other health studies, will allow EPA to assess the potential health risks of these substances.

F. Gordon Hueter
Director
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ABSTRACT

At various times after exposure of the adult mouse to single or repeated (multiple) doses of cerium (Ce) citrate, the tissue/organ distribution and effects of Ce on selected behavioral parameters (ambulations and rearings in the open field, "hole-in-board" exploratory behavior, passive and active avoidance learning, and an aspect of social behavior) were investigated. Effects of Ce on the gravid and lactating female and on parameters of embryonic, fetal and postnatal development and behavior of offspring of Ce exposed dams also were studied.

Although intragastric (i.g.) administration of Ce results in gastritis and enteritis, little Ce is absorbed from the gut. Even at levels as high as the LD_{25}, i.g. administered Ce had no significant effect on the open field or exploratory performance of the adult mouse.

At short time intervals after a single subcutaneous (s.c.) dose of Ce, systemic distribution of Ce was substantial. Open field and exploratory behaviors were significantly depressed up to 24 hours post administration and behavioral alterations correlated with tissue/organ Ce levels. An inverse relationship between behavior and brain and lung Ce levels was found. Brain levels correlated most strongly with behavior. Although the lung accumulated substantial quantities of Ce, histological examination revealed no abnormalities. The spleen contained the highest Ce concentration of any tissue examined and its Ce level was the only one positively correlated with behavior. There was no evidence of gastritis or enteritis in s.c. dosed animals.

Cerium induces fatty degeneration of the liver. Histological evidence of liver damage and regeneration was found in the animals used in the s.c. open field study. Thus, hepatotoxicity may have been involved in the observed behavioral alterations.

Activity wheel performance, a measure of general activity was depressed significantly by a single dose of Ce at the s.c. LD_5 or LD_{25} levels. The most depressed scores occurred during the 24 hour period following Ce administration. Ce also increased latency in both trials of the passive avoidance learning task although learning of the task was not affected.

A single s.c. dose of Ce at the LD_5 and LD_{25} levels had no effect on two-way active avoidance learning. However, at the LD_{25} level the activity component of this task was depressed, consistent with the open field, activity wheel and passive avoidance results.

Although mobility was depressed, Ce had no dramatic effect on the social behaviors investigated.

Cerium accumulates mainly in the spleen, liver and skeleton following s.c. exposure to repeated (multiple) doses of Ce (20 mg Ce/kg/dose) and no decrease in Ce levels was observed in these tissue for up to 98 days after the last dose. The Ce levels of the liver, kidney, pancreas, testes and possibly stomach and duodenum appeared to plateau after the fifth-sixth dose. Spleen and bone continued to accumulate Ce even after exposure was ended. Blood and brain levels were very low (counting rates near background).

Repeated exposure to Ce significantly depressed ambulations and marginally depressed explorations. These effects did not correlate with tissue/organ Ce levels (Ce was not detectable in the brain).

Exposure to Ce on day 12 of gestation resulted in significantly depressed weight and rate of growth of the neonate in depressed rearings in adult offspring.

Regarding maternal behavior, offspring retrieval data indicate that pups exposed to Ce in utero (day 7 and day 12) are retrieved in preference to control pups. Conceivably, Ce pups were preferentially retrieved because they were less active and lighter in weight than control pups.

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CONTENTS

Foreword iii
Abstract iv
Figures viii
Tables ix
Acknowledgment x

Introduction 1
Cerium 1

I. Lethality Studies 2
Materials and Methods 2
Results 3

II. Tissue/Organ Uptake and Distribution Studies 4
A. Acute Exposure 4
Materials and Methods 4
Results 5
B. Repeated (Multiple) Dose Exposure 6
Materials and Methods 6
Results 7
C. Tissue Distribution During Development: Mother, fetus, and offspring 8
Materials and Methods 8
Results 9

III. Behavioral Studies 11
A. Acute Exposure Studies 11
1. Open Field Behavior 11
Materials and Methods 11
Results 12
2. Exploratory Behavior 13
Materials and Methods 13
Results 13
3. Activity Wheel 14
Materials and Methods 14
Results 15
4. Passive Avoidance 15
Materials and Methods 16
Results 16

5. Active Avoidance 17
Materials and Methods 17
Results 18

6. Social Behavior 18
Materials and Methods 18
Results 19

B. Repeated (Multiple) Exposure Studies 19
1. Open Field and Exploratory Behavior 19
Materials and Methods 19
Results 20

C. Developmental Studies 20
1. Neonatal Studies 20
Materials and Methods 21
Results 21

2. Maternal Offspring Retrieval Latency 22
Materials and Methods 22
Results 23

3. Adult Offspring Behavior 23
Materials and Methods 23
Results 24

IV. Biochemical Studies: The Subcellular Distribution of Ce in the Liver 25
Materials and Methods 25
Results 29

V. Conclusions 30
1. The Tissue/Organ Distribution and Alterations in Open Field and Exploratory Behavior Following Acute Exposure to Ce . . . 30

2. Activity Wheel Study 32

3. Passive Avoidance 32

4. Active Avoidance Behavior 32

5. Social Behavior 32

6. The Tissue/Organ Distribution and Alterations in Open Field and Exploratory Behavior Following Exposure to Repeated Doses of Ce 33

7. Neonatal and Adult Offspring Studies 34

8. Maternal Behavior 34

9. Liver Subcellular Distribution of Ce 34

FIGURES

Number		Page
1	Effects of Cerium (citrate) on open field and "hole-in-board" exploratory behavior. Subjects (adult male mice) were observed (automated open field or automated exploratory apparatus) at 4 hrs., 1, 3 or 7 days post i.g. or s.c. administration of either $CeCl_3$ (complexed 1:3 with sodium citrate) or sodium citrate solution (controls). Total ambulations, rearings and explorations (means $\pm$ s.d.) for 5 minute observation periods are shown. Treatments were: (■) Ce at the 7 day LD_{25} level (i.g. = 1163 ppm Ce; s.c. = 173 ppm Ce). (◆) Ce at the 7 day LD_5 level (i.g. = 1000 ppm Ce; s.c. = 136 ppm Ce). (●) Sodium citrate (i.g. = 6140 ppm sodium citrate, s.c. = 917 ppm sodium citrate)	40
2	Effects of Cerium (citrate) on wheel running performance. One hour before light onset on Day 0, subjects (adult male mice) received either a single s.c. dose of Ce at the LD_5 or LD_{25} level or sodium citrate solution (controls). Total wheel revolutions were recorded continuously during the dark phase of the light cycle (lights on at 7:00 p.m., off at 7:00 a.m.). Symbols: ▲ sodium citrate; ● Ce at the 7 day LD_{25} level; ⊖ Ce at the LD_5 level.	41
3	Activity board. The field was painted a light, flat grey. The circles and radii were dark grey. The center circle had a diameter of 5.5 cm	42
4	Circular runway. The runway was enclosed by concentric aluminum walls. The floor of the runway was painted a light, flat grey and the lines, dark grey	43

TABLES

Number		Page
1	Effect of Increasing Ce Dose on 7 Day Viability of Gravid and Nongravid Animals	44
2	Ce Lethal Dose Levels as Determined by Linear Regression Analysis of 7 Day Viability	45
3	Tissue/Organ Ce Concentration in µg Ce/g (Wet Weight) Tissue (PPM) - Acute Exposure	46
4	Analyses of Variance of Tissue/Organ Concentrations of Cerium - Acute Exposure	48
5	Tissue/Organ Ce Concentrations in µg Ce/g (Wet Weight) Tissue (PPM) - Repeated Exposure	49
6	Dam and Embryo/Fetal Tissue Ce Distribution Following Day 7 Administration (PPM)	52
7	Dam, Placental and Fetal Tissue Ce Distribution Following Day 12 Administration	53
8	Dam and Offspring Tissue Ce Distribution Following Day 2 Post Partum Administration	54
9	Analysis of Variance of Open Field Behavioral Measures - Acute Exposure	55
10	Analysis of Variance of Behavioral Measures - Acute Exposure via the s.c. Route	56
11	Correlations Between Tissue/Organ Ce Levels and Ambulations and Rearings - Acute Exposure	57
12	Cell Means and Standard Deviations for Wheel Revolutions Recorded Four Hours After Ce or Citrate Administration - Acute Exposure	58
13	Effects of Ce on Mouse Open Field and Exploratory Behavior - Repeated Exposure	59
14	Liver Subcellular Ce Distribution at 1, 3 and 7 Days Post Administration (Percent of Ce Recovered)	60
15	Analysis of Variance of Liver Subcellular Ce Distribution	61

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INTRODUCTION

CERIUM

Cerium (Ce)-based compounds have been investigated as potential replacements for lead-based antiknock agents in fuels for internal combustion engines (1,2). If adopted for this purpose, Ce and certain Ce compounds might rapidly become widely distributed in the environment. The bioavailability of such substances, especially in the case of protracted exposure, could have adverse biologic effects, especially to humans. Cerium has been identified in "normal" ambient air (3), however, the literature on its toxicological effects is relatively limited (e.g. 4-13). Research on this element and its compounds has established that Ce accumulates in the liver (6), alters the endoplasmic reticulum and induces fatty degeneration (5,9,12) and suppresses the activity of drug-metabolizing enzymes (8,12,13). Cerium is absorbed through the nasal membranes into the general circulation (14) and accumulates in the lungs (15). Although Ce may be of low overt toxicity (15,16), its tissue distribution and toxicity may vary with the nature (acute, chronic) and level of exposure (10,11) and route of administration (10,11,16). Also, covert health effects cannot be discounted. For example, Ce may effect the nervous system thereby altering behavior (10,11,17).

Scope of the Research

The overall objective of this project was to investigate potential toxic effects of acute and chronic exposure to Ce employing the mouse as a model mammalian system. In the adult mouse, the tissue/organ distribution of Ce was determined at various times after exposure to a single dose or repeated (multiple) doses of $CeCl_3$. In conjunction with these studies, the effects of Ce exposure on selected behavioral parameters also were examined. Open-field and exploratory behaviors, passive and active avoidance learning, and an aspect of social behavior were investigated. Another major focus of this research was the examination of the effects of Ce on the gravid female mouse and on the embryonic, fetal and postnatal development of the offspring of Ce exposed dams. Cerium tissue/organ distribution and various maternal and offspring developmental behaviors were investigated in these studies. In the adult studies, statistical correlations between tissue/organ Ce levels and various behavioral measures were examined to gain insight into the basis of the relationships between the two sets of data.

In addition, subcutaneous administration of 136 mg Ce/kg (LD_5) was found to induce fatty infiltration of the liver. However, this effect was transient: the fatty infiltration regressed with time while the liver Ce levels remained elevated. The nature of this phenomenon was probed in studies of the subcellular distribution of Ce in the liver as a function of time.

I. LETHALITY STUDIES

Lethality parameters were established for Ce (citrate) administered intragastrically (i.g.) and subcutaneously (s.c.). The i.g. route was selected for its environmental relevance and the s.c. route because it should provide better dosage control. In addition the s.c. route is related to the transdermal route and, therefore, has environmental relevance.

MATERIALS AND METHODS

Animals

Six to 8 week old (27-33 gm) random bred Swiss-Webster mice (ICR strain, West Seneca Laboratories, West Seneca, NY) of both sexes served as subjects. The animals were housed 5/cage in suspended wire cages. Food (Agway 1000 Rat and Mouse Maintenance Diet) and tap water were freely available.

Chemicals

Cerium chloride (anhydrous powder, 99.9% pure on a rare earth oxide basis) was obtained from Alfa Products - Ventron Corporation (Danvers, MA). ^{141}Cerium (chloride or nitrate; 2.5 Ci/g) was obtained from New England Nuclear (Boston, MA). The nitrate form was converted to the chloride by drying in a stream of N_2, redissolving in 6N HCl and drying with N_2 twice and finally redissolving in 0.5N HCl (the procedure recommended by the supplier). Sodium citrate, reagent grade, was obtained from Baker Chemical Co. (Phillipsburg, NJ).

$CeCl_3$ is insoluble at physiological pH. Thus, a 1:3 $CeCl_3$:sodium citrate complex was prepared and adjusted to pH 7.4 with NaOH. Sodium citrate control solutions were adjusted to pH 7.4 with HCl. Solutions for injection were prepared by dilution of stock solutions and autoclaving at 121°C at 20 psi for 30 minutes.

Dosing

Lethality was assessed via administration of logarithmically spaced doses (at a constant volume of 0.2 ml/30 gm) to groups of 10 (or more) randomly selected animals. Control groups received sodium citrate (pH 7.4) at a concentration comparable to that in the highest Ce dose investigated.

Protocol

The number of deaths in each group were recorded through day 7 post administration. From such data, the lethal dose percentiles (LD_{xx}) and confidence intervals were obtained by the method of probit analysis as described by Finney (18) using the Isobol computer program of Gessner and Cabana (19).

RESULTS

For males, the LD_{50}, LD_{25} and LD_{5} (with 95% confidence intervals) levels were: 1291 (1198-1440), 1163 (1017-1250) and 1000 (743-1104) mg Ce/kg, respectively, for the i.g. route of administration and 205 (181-241), 173 (140-195) and 136 (86-160) mg Ce/kg, respectively, for the s.c. route.

Nongravid and gravid (day 12 of gestation) females received 68 to 318 mg Ce/kg. Day 7 viability and lethal dose levels (calculated by probit analysis) for the s.c. route of administration are summarized in Tables 1 and 2, respectively.

II. TISSUE/ORGAN UPTAKE AND DISTRIBUTION STUDIES

The tissue distribution of Ce was investigated in adult males and females, in the developing fetus and in offspring of Ce exposed dams.

A. ACUTE EXPOSURE

MATERIALS AND METHODS

Animals

As described above in the lethality studies, two hundred and forty 6 to 8 week old male mice served as subjects.

Chemicals

Chemicals and dose preparation were as described in the lethality studies cited above.

Dosing

Two dose levels were selected for investigation, the LD_5 and LD_{25} (calculated from the LD_{50} data, above). The actual doses employed were: (i) 1000 (LD_5) and 1163 (LD_{25}) mg Ce/kg (ppm) and 6140 mg sodium citrate/kg (controls) for the i.g. route and (ii) 136 and 173 mg Ce/kg (ppm) and 917 mg sodium citrate/kg (controls) for the s.c. route. Doses were administered in a volume equivalent to 1% (v/w) of the subject's body weight (bw). In each case, the control dose contained the same amount of sodium citrate as the respective LD_{25} dose. The injection solution contained 8μCi ^{141}Ce/animal. Animals dosed i.g. were lightly etherized prior to administration for ease and consistency of dosing.

Protocol

Four post administration times of observation were selected (4 hr.; 1, 3 and 7 days) to monitor the time dependent changes in tissue/organ Ce levels and behavior.

Immediately following behavioral observation, the Ce animals were sacrificed by overdosing with ether. Blood was obtained by cardiac puncture and the liver, spleen, kidneys, lung, stomach, duodenum, skeletal muscle (thigh), pelt, cerebrum, cerebellum and brain stem were removed. The tissues/organs were weighed and Ce levels were quantified by gamma scintillation spectrometry.

Tissues from several mice in each of the day 7 treatment groups were prepared for histopathological examination. Representative samples of skin, lungs, kidneys, liver, stomach, intestines, pancreas, spleen, heart, skeletal muscles, brain and testis were fixed in neutral buffered 10% formalin and embedded in paraffin. Sections 6μ thick were stained with hematoxylin and eosin or the Gomori trichrome stain.

RESULTS

Tissue/organ Ce levels in μg Ce/g wet weight tissue (ppm) are presented in Table 3. Dose levels can be expressed in two ways: (i) in terms of their relative toxicities (lethal dose percentiles), which we believe is appropriate for analysis of the behavioral results, or (ii) in terms of the actual amount administered (mg Ce/kg); which we believe is more appropriate for the analysis of tissue/organ concentrations of Ce since such concentrations are dependent, in part, on the actual dose of Ce. The latter procedure yields a design with 4 dose levels of Ce (1163, 1000, 173 and 136 ppm), with 2 levels nested within each route (i.g. = 1163, 1000 ppm; s.c. = 173, 136 ppm). Tissue/organ concentrations of Ce were, therefore, analyzed as a 4 (time) by 2 (route) by 4 (dose) multivariate design with dose nested within route. The results of this analysis are presented in Table 4.

All tissues/organs exhibited significant main effects of time and/or route. The liver, kidney, lung, muscle, cerebrum and brain stem also showed an effect of dose; but only within the s.c. route. Many tissues exhibited a time by route interaction; but only lung exhibited a dose by time interaction and only via the s.c. route. The strongest effect by far was that of route. This was anticipated (20) and largely reflects the poor uptake of Ce via the i.g. route (Table 3) and the quantitative differences in the actual doses administered via each route. The time effects were due to the time dependent decrease in tissue Ce [except in the spleen where Ce peaks between 1 and 3 days post administration (Table 3)]. The significant dose effects via the s.c. route were due to higher tissue/organ Ce levels in animals receiving the higher Ce dose. The absence of such effects in some tissues may be accounted for by the high variability encountered at the doses employed. The time by route and time by dose interactions within the s.c. route may be attributed to route/dose pharmacokinetic differences.

Histopathology

Gastritis and enteritis were evident in all i.g. dosed animals examined on day 7 post Ce administration. In these animals, the stomach and the duodenum manifested focal hemorrhages and necrosis of the mucosa. Homogeneous conglomerates of eosinophilic substances were found within the necrotic mucosa. In addition, conspicuous polymorphonuclear cell infiltration was observed in the gastric and duodenal lamina propria and submucosa, especially in those areas adjacent to the necrotic zones.

There was no evidence of gastritis and/or enteritis in the s.c. dosed animals. However, the livers of these animals, especially at the higher dose level, exhibited focal mid-zonal necrosis. Regenerative changes, characterized by a moderate number of mitotic figures in hepatocytes, also were observed.

Hypertrophy, reticuloendothelial hyperplasia and hyperactive lymphoid follicles were observed in the spleens of Ce treated animals, irrespective of route of administration. All other tissues examined revealed no significant changes.

B. REPEATED (MULTIPLE) DOSE EXPOSURE

MATERIALS AND METHODS

Animals

Six to 8 week old male mice served as subjects.

Dosing

On day zero, each subject was administered (s.c.) either 20 ppm Ce and 0.3 μCi ^{141}Ce in a volume equivalent to 1% (v/w) of body weight or an equivalent volume of sodium citrate solution containing 105.3 mg Na citrate/kg.

Protocol

On day 3, 10 animals were randomly selected from each group and observed in the open-field and exploratory apparatuses. Following observation, the Ce animals were sacrificed by overdosing with ether. Blood was obtained by cardiac puncture and the liver, spleen, kidney, pancreas, lung, stomach, duodenum, testes, skeletal muscle, bone, cerebrum, cerebellum and brain stem were collected and weighed. Tissue/organ Ce concentrations were quantified by gamma scintillation spectrometry. The remaining animals, which had not been selected for observation, were injected again, as on day zero, except the injection site was shifted to a different area of the back to avoid excessive irritation. This procedure was repeated for a total of 10 injections; 1 every 3 days for 30 days. In all cases, animals were observed 3 days after receiving the last dose of a sequence. The maximum total Ce exposure was 200 mg Ce/kg which is approximately equal to the single dose 7 day LD_{50} level: 205 (181-241) mg Ce/kg.

To provide data on tissue/organ Ce retention, a number of additional animals were carried through the entire dosing regimen and sacrificed in 2 groups at 42 and 98 days after the last injection.

The data were analyzed using a one way (exposure) multivariate analysis of variance (ANOVA) for tissue/organ Ce levels and a two way (exposures by treatment) multivariate ANOVA for the behavioral measures. Relationships

between behavior and tissue levels were investigated using a step-wise multiple regression analysis. ANOVAs and the multiple regression analyses were performed using the Multivariance Computer Program of Finn (21). Locus of effects was investigated using Tukey's Honestly Significant Difference Procedure performed by The Statistical Package for the Social Sciences (SPSS), Subprogram Oneway (22).

In certain cases, we discuss effects which did not attain generally accepted levels of significance ($p < 0.05$). However, in a study such as this, focused on biological effects resulting from exposure to a potentially toxic substance, we believe it would be unwise to completely disregard marginal effects with probabilities between 90 and 95%. Therefore, we have noted such effects and their probabilities; but have not declared them significant.

For some unknown reason, a number of animals in this study exhibited Ce levels in certain tissues/organs that were considerably higher (3-200 times) than those of the majority of animals in their treatment groups. Due to the uncertain nature of this phenomenon, and because inclusion of such data in the analyses could obscure typical effects, even if such tissue/organ Ce levels arose naturally, we have chosen to eliminate these animals from the analyses. Thus, the data from 12 of the 100 Ce exposed animals were excluded from analysis.

RESULTS

The tissue/organ Ce levels and the number of animals remaining in each group are presented in Table 5. The multivariate ANOVA showed that, across all tissues, there was a significant difference in Ce concentration across exposures ($F = 2.27$, d.f. = 126/510.92, $p < 0.001$). The univariate F-ratios showed that this was also true ($p < 0.05$) for all tissues individually, except blood, cerebellum and brain stem. For the most part, the effect was one of increasing Ce tissue/organ levels with increasing number of exposures. Muscle levels were highly variable and may reflect some direct absorption of Ce via migration from the injection sites.

In the animals excluded from analysis because of inordinately high tissue/organ Ce levels, the pancreas and kidney and, to a lesser extent, the stomach and intestine were involved (the remaining tissues exhibited "typical" Ce levels). Since a number of different tissues/organs in the same animal were affected, contamination or counting error appeared to be ruled out. Presently, this phenomenon cannot be explained. Conceivably, it could have resulted from faulty administration such that, for one or more injections, either the peritoneum or a vein was inadvertently penetrated thereby effectively changing the route of administration. Alternatively, some pathological change which dramatically altered the rate of Ce uptake by certain tissues/organs may have been the cause. Remarkably, the behavioral data for these animals were indistinguishable from those of the other animals within their groups.

C. TISSUE DISTRIBUTION DURING DEVELOPMENT:

Mother, fetus, and offspring.

MATERIALS AND METHODS

Animals

Female mice (27-33 gm; avg. 30 gm) were bred to competent males. Observation of a vaginal plug defined day 0 of pregnancy. Pregnant females were housed individually on wood chip bedding and allowed access to food and water *ad libitum*. Mice that were to be used in *post partum* experiments were allowed to deliver and their litters immediately culled to 6 pups: 3 males and 3 females. Day of delivery was defined as day 1 *post partum*.

Dosing

On days 7 or 12 of gestation or day 2 *post partum*, randomly selected mothers were injected s.c. either with 80 ppm Ce (7 day LD_1 dose) containing 5 μCi ^{141}Ce/ml, or an equivalent volume (0.2 ml/30 gm bw) of sodium citrate solution (controls) containing 424 mg sodium citrate/kg.

Protocol

A complete cross-fostering design was employed to distribute the offspring of mothers receiving Ce or citrate on days 7 or 12 of gestation to foster mothers having received either Ce or citrate; producing three offspring test groups: Ce x Ce [denoting offspring's *in utero* exposure background first x their post natal (lactational) exposure], Ce x citrate (Cit) and Cit x Ce.

Following Ce administration on day 7 of gestation, dams were sacrificed by cervical dislocation on days 8, 9, 10 and 12 of gestation and days 7, 12 and 21 *post partum*. Following Ce administration on day 12 of gestation, dams were sacrificed on days 13, 14, 15 and 17 of gestation and days 7, 12 and 21 *post partum*. Following Ce administration on day 2 *post partum*, dams were sacrificed on days 3, 4, 5, 12 and 21 *post partum*. The ^{141}Ce levels of maternal blood, liver, kidney, spleen, gastrocnemius muscle, uterus, cerebrum, cerebellum, and ovary and the embryos, fetuses and placentas were quantified by gamma scintillation spectroscopy.

On days 3, 4, 5, 12 and 21 *post partum*, pups exposed to Ce via suckling only (day 2 *post partum* Ce administration to dams) were sacrificed by overexposure to ether and whole body ^{141}Ce levels were determined. On days 7, 12 and 21 *post partum*, the ^{141}Ce level of blood, liver, kidney, cerebrum and cerebellum was measured in representative numbers of pups (randomly selected) from all Ce exposure groups (Ce x Ce; Ce x Cit; Cit x Ce).

RESULTS

The ^{141}Ce levels of maternal tissues and the developing embryo/fetus, following administration on day 7 of gestation, are shown in Table 6. Cerium levels were highest in all maternal tissues 1 to 2 days after Ce administration and then declined through the remainder of the gestation period. The highest Ce level observed was in liver (333 ± 23 ppm) 2 day post administration. The Ce levels in maternal tissues were of the order: liver > spleen > lung > kidney. Maternal brain Ce levels were low (less than 0.3 ppm) at all times of observation. Cerium levels in the developing organism were highest 1 day post administration (5.71 ± 1.22 ppm) and then declined 6 fold over the succeeding 4 days.

Following partuition, maternal tissue Ce levels continued to decline or remain relatively constant except in the uterus and spleen. During the early suckling period, uterine Ce levels increased 15 fold (from 1.34 ± 0.25 to 18.4 ± 2.2 ppm on day 7 post partum), and then declined through the late suckling period (to 2.82 ± 0.74 ppm on day 21 post partum). Ce levels in spleen increased 4 fold from early to late suckling (93.1 ± 31.6 to 267 ± 41 ppm from day 7 to day 21 post partum). During early suckling, Ce levels in maternal tissues were of the order: liver > spleen > uterus > kidney. During later suckling, maternal tissue Ce levels were of the order: spleen > liver > kidney > uterus.

Cerium was below the level of detectability (limit of detection < 0.05 ppm) in the individual tissues selected for examination of offspring of all treatment groups from parturition up to 21 days post partum.

Maternal, ^{141}Ce levels, following administration on day 12 of gestation, are shown in Table 7. Cerium levels peaked in all maternal tissues except spleen 1 to 2 days post Ce administration. Spleen Ce levels increased 3 fold, from 98.0 ± 52.3 to 340 ± 120 ppm, from 1 to 5 days post administration. On day 1 post administration, the Ce levels of maternal tissues were of the order: liver > spleen > lung > kidney. However, by day 5 post administration, spleen levels were approximately twice those of the liver with spleen > liver > lung > kidney.

Placental Ce levels were approximately 7 fold greater than those of the maternal blood 1 day post administration and were approximately 14, 8 and 32 fold greater, respectively, on days 2, 3 and 5 post administration. Cerium levels in the fetus were always undetectable (less than 0.05 ppm).

Following parturition, maternal tissue Ce levels continued to decline or to remain relatively constant, except for the spleen and uterus. The Ce level of the spleen increased 2 fold, from 167 ± 28 to 389 ± 48 ppm, between days 7 and 21 post partum. During this period, the Ce level of the uterus increased 4 fold from 17.5 ± 3.8 to 79.9 ± 18.3 ppm. Cerium was undetectable in the tissues of offspring of all treatment groups exposed to Ce on day 12 of gestation from parturition to day 21 post partum.

^{141}Ce levels in maternal tissues and offspring, following administration on day 2 post partum, are shown in Table 8. Cerium levels peaked in all dam tissues 1 to 2 days post administration and declined or remained essentially constant thereafter. The Ce levels in maternal tissues were of the following order: spleen > liver > uterus > lung > kidney.

Neonates had measurable whole body Ce levels up to three days post maternal Ce administration. However, Ce was undetectable in fetal blood, liver, kidney, cerebrum and cerebellum even though mean whole body content measured 0.12 ppm $\pm$ 0.02 ppm.

III. BEHAVIORAL STUDIES

Effects of exposure to acute and repeated doses of Ce, via the i.g. or s.c. routes, on selected behavioral parameters were investigated. These data were correlated with those of the tissue distribution study, previously described, in an attempt to gain insight into mode of toxic action of Ce.

A. ACUTE EXPOSURE STUDIES

1. Open Field Behavior

General activity in the open field encompasses dimensions of emotionality and exploratory behavior (23). Psychopharmacological and toxicological research have established the utility of open field behavioral measures as sensitive indices of behavioral effects of drugs (e.g. 24, 25). We have utilized the open field to determine effects of acute Ce exposure on general activity levels of the adult mouse as a function of route of exposure (s.c. vs i.g.), dose (LD_5 vs LD_{25}) and time of observation.

MATERIALS AND METHODS

Animals

The subjects were the same animals used for the acute tissue distribution study described previously.

Dosing

The animals were dosed as described above in the acute tissue distribution study.

Apparatus

A Lehigh Valley Electronics (Fogelsville, PA) Model 1497 automated open field was employed.

Protocol

The animals were observed in the open field at 4 hr.; 1, 3 and 7 days post administration. Each subject was placed initially in the central circle (diameter = 15 cm) of the field and observed for 5 minutes. The dependent measures (which we view as providing an index of general activity) were total ambulations (automatically recorded) and total rearings (visually recorded). A rearing was scored each time the subject raised both forepaws

off the floor in the absence of grooming behavior. This experimental design generated a 2 (route of administration) by 3 (treatment: Ce at the LD_5 or LD_{25} levels or citrate) by 4 (time of observation) matrix with 24 cells. Each cell contained 10 animals.

RESULTS

Analysis of the results employing a 2 (route of administration) by 3 (dose) by 4 (times of observation) multivariate ANOVA (Table 9) indicated that all main effects and interactions were highly significant ($p < 0.05$ for all multivariate F-ratios) except for the route by dose interaction ($p > 0.05$). All univariate F-ratios also were significant for both ambulations and rearings except the route by dose effect and the time by dose effect for rearings.

Graphic representation of the data (Figure 1) indicated that the behavioral effects were limited to the s.c. route of exposure. Since this would greatly simplify interpretation, each route was reanalyzed in a separate 2 way analysis of variance. As suggested in Figure 1, the i.g. analysis yielded no significant effects. However, the s.c. analysis (Table 10) yielded highly significant effects of time, dose and a time by dose interaction.

To further specify the locus of simple effects, the Tukey Honestly Significant Difference Test (22) was applied to these data. This analysis revealed that, via the s.c. route: (i) the main effect of time was primarily accounted for by the decreased activity of the 4 hour groups versus all other times of observation; (ii) the significant dose effect was largely due to the decreased activity associated with the high Ce dose and (iii) the time by dose interaction was largely accounted for by the fact that the 4 hour high dose Ce animals were significantly less active than the animals at all other doses, at all other times. The balance of the interaction can be accounted for by the fact that, at 4 hours, the low Ce dose animals were less active than controls and, that at 1 day, the high Ce dose animals were less active than controls. Thus, the behavioral effects are reflected in reduced activity (ambulations and rearings). These effects are largely limited to the s.c. route of administration and the higher dose of Ce at short time intervals post-exposure.

Relationship Between Open Field Behavior and Tissue/Organ Ce Levels: The data base for these analyses was derived from the Ce treated animals only. In the overall test, a significant association between tissue/organ Ce levels and ambulations and rearings (Table 11) was found. Examination of the individual correlation coefficients revealed strong inverse correlations between the behavioral measures and lung, kidney, cerebrum, cerebellum, liver and blood Ce levels.

Because significant behavioral effects were found for the s.c. route of administration, the correlation analysis was repeated for each route separately. Within the i.g. route, there was no significant overall association ($F = 1.25$, d.f. = 24/132, N.S.) nor any significant individual

correlation coefficients. However, there was a significant overall association within the s.c. route (Table 11). The strongest correlations with the behavioral measures within this route were inverse correlations with the brain regions followed by the lung, stomach, blood, intestine and kidney.

2. Exploratory Behavior

Effects of acute Ce exposure on "hole-in-board" exploratory behavior of adult mice were investigated as a function of route of exposure (s.c. vs i.g.), dose (LD_5 vs LD_{25}) and time of observation. This task requires little physical movement on the part of the subject and, thus, provides a measure of exploratory drive which is not greatly confounded by general activity.

MATERIALS AND METHODS

Regarding the number of animals, routes, levels of exposure and times of observation, the design of this study was identical to that of the open field study described above. The subjects investigated in this study were treated as previously described in the acute tissue distribution study. However, they received unlabeled Ce only and tissue/organ Ce levels were not measured.

Apparatus

The exploratory apparatus consisted of a circular runway, 5.7 cm wide, formed from two concentric walls with an O.D. 34.3 cm. In the outside wall were four holes centered 90° apart and 5.1 cm above the floor. Photocells were arranged so that each time the subject put its nose into a hole the event was counted. The runway was specifically designed to minimize the general activity component of this behavior and maximize the exploratory component.

Protocol

A randomly selected subject was placed in the runway. The measure of exploratory behavior was the number of hole entries per subject automatically recorded over a 5 minute period.

RESULTS

The results of this study are presented in Figure 1. In the overall ANOVA, significant main effects of time ($F = 8.59$, d.f. = 3/216, $p < 0.001$) and route ($F = 8.48$, d.f. = 1/216, $p < 0.01$) and significant time by route ($F = 3.96$, d.f. = 3/216, $p < 0.001$), and route by treatment ($F = 3.29$, d.f. = 2/216, $p < 0.05$) interactions were found. However, as in the open field study, when data were analyzed separately for each route, the i.g. route yielded no significant behavioral effects. Via the s.c. route, significant main effects of time ($F = 11.44$, d.f. = 3/108, $p < 0.001$) and treatment ($F = 5.18$, d.f. = 2/108, $p < 0.01$) and a significant time by treatment ($F = 4.91$, d.f. = 6/108, $p < 0.001$) interaction were observed. Tukey's Honestly Significant Difference Procedure (22) revealed that, within the s.c. route, the 4 hour time period produced significantly fewer exploratory responses than all other times and the LD_{25} treatment produced a lower

response rate than citrate alone (control). However, the LD_5 treatment did not differ from the LD_{25} or citrate treatments. Thus, a similar pattern emerged for exploratory and open field behavior: a single dose of Ce at a moderately high level (LD_{25}) significantly depressed behavior, in a transient manner, shortly after administration (4 hr.) while a lower dose (LD_5) produced no significant effects. Recovery to control levels of behavior was observed by 24 hours after administration.

3. Activity Wheel

This study was undertaken to investigate the effects of acute Ce exposure on a measure of long-term general activity. The data complement the short-term activity data provided by the open field study described previously.

MATERIALS AND METHODS

Animals

Six to 8 week old male mice were acclimated to the home cage for one week prior to Ce administration.

Dosing

Ce citrate was administered to the experimental animals via the s.c. route as described above for the open field study. Ten animals received a single dose at the LD_5 level (136 ppm Ce) and 10, a single dose at the LD_{25} level (173 ppm Ce). Ten control animals received sodium citrate only. The animals were injected 1 hour before light onset on Day 0 of the experiment and were returned to their home cages immediately post administration.

Apparatus

A plexiglass activity wheel with attached home cage [modeled after the standard Wahmann (LC34) mouse activity wheel] was employed. The dimensions of the home cage were 23.8 x 17.5 x 12.5 cm. The activity wheel had a diameter of 20.0 cm and was 6.8 cm wide.

Protocol

Total wheel revolutions were recorded continuously during the dark phase of the light cycle (lights on at 7:00 p.m., off at 7:00 a.m.). Scores (wheel revolutions) for each of 7 days post administration constituted the behavioral measure.

Two separate analyses were performed on the activity wheel data. Total wheel revolutions at 4 hours post administration were subjected to a one-way ANOVA. This time was chosen in order to evaluate the short-term effects of Ce administration. In order to evaluate the long-term effects of Ce administration, total wheel revolution scores for each of the 7 days post administration were analyzed by a one-way multivariate ANOVA for repeated

measures. The injection groups comprised three levels of the same factor (Treatment). Wheel revolution scores for each of the 7 days were treated as multiple intercorrelated responses from the same subjects (Days). To test the various effects, new variables were formed by combining the 7 original "day" scores. The treatment effect was obtained by summing the individual day scores and testing the resultant variable for differences among treatments. Trend analyses across time were obtained by combining the original variables using orthogonal polynomial coefficients to obtain linear, quadratic and cubic combinations. These combinations were tested for differences from zero which occur only if the trend (linear, quadratic, cubic) is significant. The treatment x days interaction was obtained by testing the same combinations (linear, etc.) for differences among treatment groups. The trends were tested in a step down fashion which provides tests of each trend free of effects due to lower order trends (e.g. cubic effect free of linear and quadratic effects).

RESULTS

The cell means and standard deviations are presented in Table 12. The one-way ANOVA revealed a significant difference in total wheel revolutions at 4 hours post administration (Day 0) between the experimental and control groups ($F = 55.56$, d.f. $= 2/27$, $p < 0.0001$). The Scheffe´ test (26) indicated that each group (LD_5, LD_{25} and control) differed significantly from the other and the activity of the animals receiving Ce at the LD_5 level was depressed the most.

A separate ANOVA examined total wheel revolutions 7 days post administration. A significant effect of treatment was found for the total wheel revolution scores ($F = 3.76$, d.f. $= 2/27$, $p < 0.04$). The significance is attributed to depressed scores of the Ce groups compared to the citrate control group. Ce animals injected at the LD_5 level had lower total revolution scores than Ce animals injected at the LD_{25} level.

Means over the 7 days were tested for linear, quadratic and cubic trends to obtain a general picture of the shape of the 7 day performance curve for each group. Significant linear ($F = 57.77$, d.f. $= 1/27$, $p < 0.001$) and cubic ($F = 16.80$, d.f. $= 1/25$, $p < 0.001$) effects were found. No significant treatment by days interaction was found. Figure 2 illustrates the trends in the daily scores of the Ce and citrate groups across the 7 day period of observation. Note the similarity in the shape of the curves for each treatment indicating lack of a Treatment x Days statistical interaction.

4. Passive Avoidance

To examine effects of acute Ce exposure on brain processes higher than those that may be predominant in general activity and exploratory behavior, passive avoidance, a measure of simple learning, was investigated. Since the passive avoidance paradigm requires little activity on the part of the subject (whose activity, as we have shown, is depressed by Ce exposure), it should provide a measure of learning that is not confounded by effects on activity level.

MATERIALS AND METHODS

Regarding the number of animals, routes, levels of exposure and times of observation, the design of this study was identical to that of the open field study previously described.

Apparatus

The passive avoidance apparatus consisted of a larger and smaller compartment separated by a (2.5 cm) hurdle. The interior of the smaller compartment (5.1 x 17.0 cm) was painted white, that of the larger (17.8 x 20.3 cm), black. Both compartments had a grid floor. That of the larger was wired to receive a scrambled shock.

Protocol

Each subject was placed into the smaller compartment of the apparatus and the time taken to cross into the larger compartment was recorded (trial 1 latency). One second after entering the larger (fear) compartment the subject was given a 0.1 mA shock which lasted until the subject crossed back into the smaller (safe) compartment. Twenty-four hours later (to allow for learning consolidation), the subject was placed back into the smaller compartment and the time required to cross into the larger compartment was recorded (trial 2 latency). A significant difference between trial 1 and trial 2 latencies (increased trial 2 latency) represents significant learning.

Analysis

The experiment was analyzed as a 3 between (time, route, dose), 1 within (trials) repeated measures design using the sum of the 2 trials to test the between effects and the difference between trials to test the within effects (all effects involving trials).

RESULTS

The analysis revealed significant main effects of time ($F = 7.89$, d.f. = 3/216, $p < 0.0001$), treatment ($F = 3.51$, d.f. = 2/216, $p < 0.03$) and trials ($F = 367.87$, d.f. = 1/216, $p < 0.0001$) as well as significant interactions of time by treatment ($F = 2.34$, d.f. = 6/216, $p < 0.03$), time by trials ($F = 7.88$, d.f. = 3/216, $p < 0.0001$) and time by treatment by trials ($F = 2.22$, d.f. = 6/216, $p < 0.04$).

Tukey's Honestly Significant Difference Procedure (22) revealed that the time effect and the time by trials effect were entirely due to shorter total latencies (trial 1 + trial 2) and smaller increases in latencies (trial 2 minus trial 1) at 4 hours than at any other time. The treatment effect was due to longer latencies for LD_{2_5} subjects than for citrate control subjects in both trials. The trials effect was due to longer latencies on trial 2 than trial 1, which demonstrates successful learning, across all subjects. Finally, the time by treatment and the time by treatment by trials effects

parallel one another and can be explained as follows. At 4 hours all treatments showed shorter total latencies across the 2 trials and smaller differences in latencies between the trials (poor learning). The LD_{25} dose showed increases in both measures through seven days whereas the LD_5 and citrate doses peaked at 1 day and declined somewhat by 7 days.

5. Active Avoidance

Two-way active avoidance has been well established as a useful measure of learning ability for a variety of species including pigeons, rodents and cats (27,28) under a variety of test conditions (29). In this procedure, a conditioned avoidance response is developed in a situation in which a signal (of 10 seconds duration) is associated with electric shock from which the animal can escape by moving to a "safe" compartment. By learning to move as soon as the signal is initiated, the animal can avoid the shock. Effects of acute exposure to Ce at the LD_5 and LD_{25} levels on a two-way active avoidance were investigated in the adult mouse.

MATERIALS AND METHODS

Animals

Thirty-nine male mice, 6 to 8 week old, served as subjects.

Dosing

Three groups of 13 animals served as subjects; receiving Ce at the 7 day s.c. LD_5 or LD_{25} levels or citrate alone (controls). The dosing procedure was the same as that of the tissue distribution study described previously.

Apparatus

An automated two-way active avoidance box was used in this study. The box was divided into two compartments by a center doorway. A shock grid of stainless steel rods comprised the floor of each compartment. Photocells were located on either side of the doorway to record the subject's crossings between the two compartments. Speakers were located at each end of the apparatus.

Protocol

Training commenced 24 hours post administration. The conditioned stimulus (CS) was a 1000 Hz tone from the speakers. The unconditioned stimulus (UCS) was a 0.2 mA scrambled shock. The CS-UCS interval was ten seconds. The inter-trial intervals varied from 30 to 60 seconds with a mean of 45 seconds. Subjects could avoid the shock by moving to the opposite compartment during the 10 second CS-UCS interval. If such a response was made, the CS was terminated and the shock was withheld. If the subject did not cross during the 10 second interval, the shock was initiated. It was

terminated when the subject crossed into the opposite compartment. Crossings were allowed at all times. Solid-state programming equipment recorded avoidance responses, escape responses and all crossings.

All subjects received 25 trials per day for five consecutive days or until they reached a learning criterion of 9 out of 10 avoidance responses. The measure of trials to criterion was the dependent learning variable. An activity score was calculated for each subject by dividing the total spontaneous crosses by the trials to criterion.

RESULTS

Trials to criterion and the activity scores were analyzed by separate one-way analyses of variance. There was no significant effect of Ce on trials to criterion ($F = 1.61$, d.f. = 2/36). However, there was a significant effect of Ce on the activity scores ($F = 3.59$, d.f. = 2/36, $p < 0.05$). Independent Student's t-tests showed that the activity scores for the LD_{2}, Ce group were significantly lower than those for the LD_{5} group. However, neither the LD_{5} nor LD_{2}, groups differed from the citrate group.

6. Social Behavior

Covert toxic reactions may be manifested as subtle alterations of complex behavioral patterns. Thus, the effects of Ce on 3 measures of murine social behavior were investigated.

MATERIALS AND METHODS

Animals

One hundred and twenty, 6 to 8 week old male mice served as subjects.

Apparatus

The apparatus consisted of a circular open field, approximately 1.2 meters in diameter, enclosed by a 46.0 cm high wall. The field was painted white and marked off in black into 49 sections of approximately equal shape and area by a series of concentric circles and radii. Each section was numbered (from 1 to 49).

Dosing

The subjects, randomly selected, received either Ce at the LD_{5} or LD_{2}, levels or sodium citrate (controls) via the s.c. route.

Protocol

The treatment groups were observed at 1 or 3 days post administration. Thus, a 3 (dose) by 2 (time of observation) experimental design was employed. This design created 6 conditions with 20 subjects (selected at random) per condition.

Animals were paired randomly within a given condition creating 10 pairs per cell. Members of the pairs were placed at opposite sides of the innermost concentric circle and were observed in the open field for a five minute period. The number of pair contacts and the total time in contact were measured as two indicators of social attraction. Also, the location of each animal was recorded photographically at 10 second intervals. From the film record, the mean distance apart was calculated as an index of social attraction. Finally, from the film record, a mobility (or activity) score was computed for each subject based upon the straight line distance between the numbered sections occupied by the subject in consecutive 10 second intervals.

RESULTS

A multivariate ANOVA revealed two significant effects, a dose effect, [LD_5 vs LD_{25} vs citrate (control), ($F = 2.34$, d.f. = 8/90, $p < 0.025$)] and a dose by day of observation interaction ($F = 2.33$, d.f. = 8/90, $p < 0.025$). Univariate analyses of the effects underlying the multivariate analysis revealed that dose affected mobility: the control (citrate) group of animals was significantly more active [$F = 4.37$, d.f. = 2/51, (Tukey, $p < 0.02$)] than either of the Ce groups (which did not differ from each other). The dosage by day of observation interaction indicated that the distance two animals maintained between themselves in the open field was affected both by the dose and the day of observation. Animals receiving the LD_{25} and, observed on Day 3 of testing remained significantly further apart [$F = 3.51$, d.f. = 5/48, (Tukey, $p < 0.01$)] than those of the other groups (which did not differ from one another or across day of observation).

B. REPEATED (MULTIPLE) EXPOSURE STUDIES

1. Open Field and Exploratory Behavior

Effects of repeated exposure to Ce on open field and exploratory behavior were studied and correlated with tissue levels of Ce.

MATERIALS AND METHODS

With regard to the number of animals, dosing conditions and times of observation, the layout of the experiment was identical to that described above for the repeated dose tissue distribution study.

Apparatus

The automated open field and exploratory apparatuses were identical to those described above in the acute behavioral studies.

Protocol

Half of the animals in each treatment group were observed first in the open field and then in the exploratory apparatus. With the other half, the procedure was reversed.

RESULTS

The results of the multivariate and univariate ANOVAs for the behavioral measures are presented in Table 13. There was a significant multivariate effect of the number of exposures, with significant univariate Fs for rearings and explorations. The multivariate treatment (Ce vs citrate) effect approached significance ($p < 0.07$) with a significant univariate F for ambulations and a nearly significant F ($p < 0.08$) for explorations. Tukey's test (22) revealed that the number of exposures effect for rearings was due to exposures 3, 5 and 6 having a higher number of rearings than did exposure 9 across both treatments. For explorations, exposures 5 and 6 were higher than exposure 10 across both treatments. The treatment effect for both ambulations and explorations resulted from depressed activity in Ce animals relative to citrate controls (ambulations: Ce=200.8, Cit=218.0; explorations: Ce=34.0, Cit=37.8).

Relationship Between Behavioral Measures and Tissue/Organ Ce Levels

The data base for this analysis was obtained from the Ce animals alone since tissue Ce levels were monitored by gamma scintillation spectrometry of ^{141}Ce. The overall test of the hypothesis of no association between the behavioral and tissue measures was accepted [$F = 1.01$, d.f. = 42/211.39, N.S. ($p < 0.46$)].

C. DEVELOPMENTAL STUDIES

1. Neonatal Studies

Although it is well known that the developing mammalian central nervous system (CNS) is particularly vulnerable to insult from environmental toxicants, essentially nothing is known about effects of Ce (compounds) on mammalian development, growth and behavior. Therefore, we undertook an investigation of effects of Ce (citrate) on the development and behavior of the mouse and the maternal/offspring unit. Our experimental approach was designed to investigate long-term effects of Ce administered at specific times during gestation and lactation on: (i) selected postnatal offspring parameters (weight, gross activity of neonate, open field behavior and simple learning), and (ii) maternal behavior (offspring retrieval latency).

MATERIALS AND METHODS

Animals

Female mice were bred and maintained as described above in the developmental tissue distribution studies.

Dosing

On days 7 or 12 of gestation or at two days post partum, groups of 20 females were injected s.c., at the 7 day LD_1 level, either with Ce citrate (80 ppm Ce) or an equivalent volume (0.2 ml/30 gm) of sodium citrate (controls).

Protocol

Immediately post partum all litters were culled to 3 males and 3 females. A complete cross-fostering design was employed to distribute the offspring of mothers receiving Ce or citrate on days 7 or 12 of gestation. The pups of mothers receiving Ce on day 2 post partum had been assigned, randomly, to foster mothers on the day of parturition.

Testing of neonates was performed on days 8 and 13 post partum. The general activity of 8 day old neonates was quantified employing an activity field (Figure 3), while that of 13 day old neonates was quantified employing a circular runway (Figure 4). The behavioral measure for both of these tasks was the number of lines crossed during one minute of observation. Each animal was tested once between 8:00 a.m. and 11:00 a.m. (dark phase of the light cycle: lights on at 11:00 a.m.; off at 11:00 p.m.). The animals were weighed immediately prior to testing and weight comprised the measure of growth. The activity scores and weights for the 3 male and 3 female neonates nursed by each mother were averaged for each sex.

Offspring behavioral performance and growth were analyzed (21) using a 2 (prenatal exposure of offspring to Ce or citrate) by 2 (Ce or citrate postnatal exposure of offspring via foster mother) by 2 (sex of offspring) factorial design. Neonate and adult parameters were analyzed separately for each time of maternal administration (days 7 or 12 of gestation).

RESULTS

The multivariate analysis of neonate scores of offspring of mothers injected on Day 7 of gestation revealed no significant differences between groups. Analysis of the neonate measures (weight and activity) of Day 12 gestational exposure groups revealed a significant multivariate main effect of prenatal exposure to Ce ($F = 4.56$, d.f. = 4/49, $p < 0.003$). The significant univariate effects underlying this multivariate effect were neonate weights on Days 8 ($F = 16.01$, d.f. = 1/52, $p < 0.0003$) and 13 ($F = 10.40$, d.f. = 1/52, $p < 0.002$) post partum. A significant interaction of prenatal and postnatal exposure ($F = 7.13$, d.f. = 4/49, $p < 0.0002$) was found for the univariate measure of Day 13 post partum weight ($F = 13.68$,

d.f. = 1/52, $p < 0.0006$). The Duncan Multiple Range test (30) was used to determine the locus of the simple effects underlying the significant main effects and interactions. These tests revealed that the main effect of prenatal exposure was accounted for by a depression in weight of offspring who were exposed to Ce *in utero* compared to offspring exposed to citrate *in utero*. Simple tests of the significant interaction, revealed that the treatment of the foster mother modified the weight gain of her offspring. These tests showed that offspring exposed to citrate prenatally have significantly lower weights by Day 13 *post partum* if reared by Cerium-treated mothers but not if reared by control foster mothers.

A 2 (maternal treatment: Ce or citrate) by 2 (sex of pup) multivariate ANOVA was performed on the weights and behavioral data of neonate offspring of mothers administered Ce or sodium citrate on Day 2 of lactation. A significant multivariate main effect of treatment was revealed ($F = 4.13$, d.f. = 4/33, $p < 0.008$). The univariate effects underlying this significant multivariate effect were reflected in significantly reduced Day 8 ($F = 16.37$, d.f. = 1/36, $p < 0.003$) and Day 13 ($F = 5.78$, d.f. = 1/36, $p < 0.022$) *post partum* weights of offspring of Ce treated mothers.

2. Maternal offspring retrieval latency

Since maternal exposure to xenobiotics can adversely affect the maternal/neonatal unit, it was of interest to investigate the effects of Ce administration to pregnant mice on a distinctive *post partum* maternal behavior, offspring retrieval.

MATERIALS AND METHODS

Animals

Mothers from each of the 4 cross-fostering conditions described above were investigated. The number in each group ranged from 5 to 10.

Dosing

Cerium dose levels and route of administration were as described above in the neonatal studies.

Protocol

Maternal offspring retrieval latency was measured on day 3 *post partum* during the 1st hour of the light phase of the light cycle. The standard litter scatter technique was employed: the mother was removed to a holding cage, the litter was removed and individual pups were placed in a randomly scattered manner in an area furthest from the maternal nest. Retrieval latency was measured as the time between replacement of the mother into the nest and the transport of the first pup to the nest. Incomplete retrievals were noted.

RESULTS

Maternal retrieval latency was analyzed by a two way (prenatal exposure vs. postnatal exposure) ANOVA, separately for each day of gestational administration. A significant main effect of prenatal exposure was obtained both for day 7 gestational exposure ($F = 4.43$, d.f. = 1/28, $p < 0.045$) and for day 12 gestational exposure ($F = 4.95$, d.f. = 1/20, $p < 0.04$). In both cases, pups exposed to Ce *in utero* were retrieved faster than pups exposed to sodium citrate *in utero*. A one way ANOVA of retrieval latencies for pups of mothers receiving Ce or citrate on Day 2 *post partum* revealed no significant differences between groups.

3. Adult Offspring Behavior

Maternal exposure to toxicants may have latent effects on offspring. Thus, it was of interest to attempt to determine if exposure of pregnant or lactating females to Ce resulted in altered behavior of their offspring in adulthood.

MATERIALS AND METHODS

Animals

Adult offspring of treated mothers from each of the 4 cross-fostering conditions described above were tested. The number in each group ranged from 10 to 22.

Dosing

Dose levels and route of administration were as described above in the neonatal studies.

Apparatus

The open field and passive avoidance apparatuses have been described previously.

Accelerating rotarod: This device consists of a steel rod 46.0 cm long and 1.9 cm in diameter which is rotated at an increasing rate by an electric motor. The rod is covered with rubber tubing for traction (total diameter of 3.8 cm) and divided into five 7.6 cm long compartments by masonite discs (25.0 cm in diameter). Each end of the bar is run on a ball race. The rod is coupled via a 100:1 reduction gearbox to a permanent magnet DC motor connected in series to a 100 ohm torgoidally wound resistor. This resistor controls a kymograph motor such that a change in speed from 2 rev/min (maximum resistance) to 50 rev/min (minimum resistance) is obtained over a period of 5.5 min. To serve as a relative measure of the speed of rotation at which a subject is driven off the rod, a gear wheel was divided into 10 equal sectors numbered 1 to 10 (maximum to minimum resistance).

Protocol

Adult offspring of Ce treated mothers were tested sequentially on day 60 *post partum* open field (general activity measured as ambulations and rearings), on the accelerating rotarod (balance and coordination) and for passive avoidance (simple learning) performances.

The open field and passive avoidance protocols were as described above.

Rotarod performance was investigated by placing either 2 males or 2 females from each litter on the stationary rod. The measure of performance was the relative speed of rotation at the time the subject drops off the rod. Each subject was given three trials. The resistor was not set back to maximum resistance after each trial.

Adult offspring data were analyzed by a multivariate factorial ANOVA using a 2 (Ce vs citrate exposed mothers) by 2 (Ce vs citrate exposed pups: *in utero* exposure) by 2 (sex of pups) design, separately for each day of gestational administration.

RESULTS

In the multivariate analysis of adult offspring measures, the only significant main effect that was found was for prenatal maternal exposure ($F = 2.45$, d.f. = 5/48, $p < 0.05$). The single underlying significant univariate effect was on rearings in the open field ($F = 7.41$, d.f. = 1/52, $p < 0.01$). *Post hoc* tests revealed that offspring exposed to Ce *in utero* on day 12 of gestation exhibited a higher frequency of rearings than offspring exposed to sodium citrate *in utero*.

A 2 (maternal treatment: Ce or citrate) by 2 (sex of pups) multivariate ANOVA was performed on the weights and behavioral data of adult offspring of mothers receiving Ce or sodium citrate on Day 2 of lactation. There were no significant differences between groups in weight or behavior.

IV. BIOCHEMICAL STUDIES:

The Subcellular Distribution of Ce in the Liver

To obtain information on the relationship of the subcellular distribution of Ce to the genesis and rapid disappearance of Ce induced fatty infiltration of the liver, the subcellular distribution of Ce was investigated.

Fatty infiltration occurs following the intravenous administration of as little as 2-4 mg Ce/kg to female rats and 7-14 mg Ce/kg to males (31, 32). It peaks 2-3 days post administration and disappears by 5-8 days post exposure. However, the biological half-life of Ce in the liver appears to be essentially infinite (33). A possible explanation for these paradoxical findings is that, somehow, Ce is sequestered in the liver and rendered biologically inactive. Sequestration might involve a time-dependent alteration in the subcellular distribution of Ce. Thus, the subcellular distribution of Ce in the liver as a function of time post administration was investigated.

MATERIALS AND METHODS

Animals

Six to 8 week old male mice served as subjects. Tap water and Charles River RMH 1000 maintenance diet were available ad libitum.

Chemicals

The source and preparation of Ce for administration have been described above. Other chemicals used in this study were:

Acrylamide and N,N-methylenebisacrylamide (Eastman Kodak Co., Rochester, NY).

Sucrose, ACS grade (Fisher Scientific Co., Fair Lawn, NJ).

Lithium lactate (Nutritional Biochemicals Corp., Cleveland, OH).

Coomassie brilliant blue G; o-Dianisidine dihydrochloride, purified B; Fast blue BB salt (diazotized-4'-amino-2',5'-diethoxybenzanilide), purified; Metrizamide; Naphthol AS-BI phosphoric acid, sodium salt; β-Nicotinamide adenine dinucleotide (B-NAD), grade III; β-Nicotinamide adenine dinucleotide phosphate, reduced form (B-NADPH), type I - chemically reduced tetrasodium salt; Nitro blue tetrazolium, grade III; Phenazine methosulfate; Succinic acid, disodium salt, hexahydrate; N,N,N',N'-Tetramethyl-ethylenediamine (TEMED); Tri-n-octylamine, grade I; Tris(hydroxymethyl)aminomethane, Trizma base, reagent grade (Sigma Chemical Corp., Saint Louis, MO).

Dosing

Each mouse received (s.c.) 136 ppm Ce (7 day LD_s) containing 5uCi ^{141}Ce in a volume equivalent to 1% (v/w) of body weight.

Protocol

Homogenization: The animals were sacrificed by cervical dislocation 1, 3 or 7 days post administration. The liver was quickly excised, weighed and minced (razor blade) on a glass plate resting on crushed ice. The mince was homogenized in 5 volumes of ice cold buffer (0.25M sucrose, 20mM Tris-HCl; pH 7.4) using two 5 second bursts of a Polytron homogenizer [set at 7 (settings range from 1-12, motor starts at 5.5)] equipped with a model PT 10 ST probe. The homogenate was filtered through 4 layers of cheesecloth into an ice cold Dounce homogenizer and further homogenized with 10 passes of the tight (A) pestle.

Differential Centrifugation: Subcellular fractions were prepared by differential centrifugation employing a modification of the procedure of Vorbeck et al. (34). A 0.5-1.0 ml aliquot of the homogenate was reserved for purposes of comparison. The remainder was fractionated into five fractions: (i) a nuclear fraction (pellet obtained by centrifugation of the total homogenate at 450g for 10 minutes); (ii) a mitochondrial fraction (pellet obtained by centrifugation of the 450g supernatant at 9500g for 10 minutes); (iii) an intermediate fraction (pellet obtained by centrifugation of the 9500g supernatant at 18,000g for 10 minutes); (iv) a microsomal fraction (pellet obtained by centrifugation of the 18,000g supernatant at 105,000g for 90 minutes); (v) a cytosolic fraction (the 105,000g supernatant). Each pelleted fraction was washed by resuspension in 2.5 - 5.0 ml of homogenizing buffer followed by: (i) recentrifugation at the low cut speed for the fraction (e.g., 450g for 10 min. for the mitochondrial fraction), (ii) recentrifugation of the resulting supernatant at the high cut speed (e.g., 9500g for 10 min. for the mitochondrial fraction) and (iii) resuspension of the resulting pellet in homogenizing buffer. The nuclear fraction was resuspended and repelleted at 450g for 10 minutes and the cytosolic fraction was recentrifuged at 105,000g for 90 minutes. All centrifugations below 105,000g were accomplished at 2°C in a Beckman model J-21 refrigerated centrifuge equipped with a model JA-20 rotor. The 105,000g centrifugation was done at 2°C in a Beckman model L ultracentrifuge equipped with a model 30 rotor.

Density Gradient Centrifugation: Subcellular fractionation by density gradient centrifugation was accomplished employing procedures adapted from the methods of Jolley et al. (35) and Khandjian (36). Aliquots of total liver homogenate and the washed, resuspended fractions obtained via differential centrifugation were diluted 1:10 with 10% sucrose gradient solution [10% (w/v) sucrose, 20mM Tris-HCl (pH 7.4), 7% (w/v) acrylamide monomer, 0.231% (w/v) bisacrylamide, 0.05 mg/ml riboflavin and 0.35 ul/ml TEMED].

Procedure A: Continuous linear sucrose density gradients were generated using a 2 chambered gradient maker. The delivery rate from the 2 chambers was kept equal by pistons which were joined together to move as one. A syringe was used to load the gradient solutions into the chambers to insure constant gradient volumes. The 10% sucrose gradient solution (described above) was placed in the outlet compartment and a 75% sucrose solution [identical to 10% solution but containing 75% (w/v) sucrose] was placed in the second compartment. Under dim illumination (to inhibit polymerization), gradients were delivered into 1 x 3.5 inch polycarbonate ultracentrifuge tubes through a length of 18 gauge stainless steel tubing which extended to the bottom of the centrifuge tubes. The total volume of each gradient was 20 ml; differing by less than 1% between gradients. The gradients were equilibrated to 4°C by overnight storage in a refrigerator. Just before use, 2 ml of cold 'cushion' [85% (w/v) sucrose], which was sufficient to fill the rounded bottom of the centrifuge tubes, was carefully injected into the bottom of each tube using a syringe and a long blunt needle. The samples were layered onto the gradients and the gradients centrifuged in a Beckman model SW-27 rotor at 27,000 rpm for either 2 or 19 hours. After centrifugation, 1 ml of distilled water was layered onto each gradient. A mold, consisting of 7 glass tubes (0.7 x 10.0 cm) maintained in a hexagonal array, was suspended above each gradient. The gradients, resting in a clear plastic rack, were slowly raised (approx. 1 cm/min.) into the molds. The gradients (excluding the 85% sucrose cushion) were polymerized by flourescent light (approx. 1 hour). Filling of the molds and polymerization were carried out at 4°C. Following polymerization, the 7 cylindrical polyacrylamide gels, resembling disc gels, were removed from each centrifuge tube. The gels were extracted from the glass tubes and either were stained for protein or reacted to detect specific enzyme activities (described below) to localize specific subcellular fractions.

Procedure B: This variation was developed to keep the nuclei from pelleting (which occurs with procedure A) in order to distinguish material bound to them from insoluable precipitates. In this procedure, a solution containing 80% (w/v) metrizamide, 7% acrylamide, 0.231% bisacrylamide, 0.05 mg/ml riboflavin and 0.35 ul/ml TEMED was used as the cushion in place of 85% sucrose. The 80% metrizamide solution has a density of 1.44 g/ml. The 0.625 x 4.0 inch polyallomar centrifuge tubes which were used required a cushion of 0.5 ml and a sample volume of 0.5 ml. These gradients were centrifuged in a Beckman model SW-27 rotor at 27,000 rpm for 19 hours. The gradients were polymerized in one piece. After removal of the gel, any Ce remaining in the tube was dissolved in 1N HCl and removed.

Subcellular fractions in the gradient gels were localized utilizing a dye-binding method for detecting protein and the visualization of colored end products of the reaction of marker enzymes. The procedures used were adapted from published methods (37,38).

Prior to protein staining or the detection of enzyme activity, the gels were dialysed against 20mM Tris-HCl (pH 7.4) for 1 hour to remove excess sucrose. For protein staining, gels were fixed in 12.5% (w/v) trichloroacetic acid (TCA) for 10 minutes and submersed in a Coomassie brilliant blue G solution [1.0 ml of 0.25% (w/v) Coomassie brilliant blue G in 12.5% TCA combined with 19 ml of 12.5% TCA just before use] for up to 2 hours at ambient temperature. The stain was replaced with distilled water (no destaining was required) and the gels were scanned at 660 nm in a Corning model 750 densitometer.

Lactate dehydrogenase (LDH) served as the enzyme marker of the cytosol. The reaction mixture for detecting LDH activity contained 0.1M lactic acid, 0.5 mg/ml NAD, 0.05 mg/ml nitro blue tetrazolium, 0.025 mg/ml phenazine methosulfate and 0.1M Tris-HCl (pH 9.2). Gels were incubated for at least 1 hour at 37°C in the dark, washed with distilled water and scanned in distilled water at 675 nm.

NADPH-tetrazolium reductase [may be related to microsomal NADPH diaphorase or NADPH cytochrome c reductase (36)] was utilized as the marker enzyme for the microsomal fraction. Activity was detected employing a reaction mixture containing 0.1 mg/ml NADPH, 0.1 mg/ml nitro blue tetrazolium and 20mM Tris-HCl (pH 7.4). Gels were incubated in the dark for at least 3 hours at 37°C, washed and scanned at 675 nm.

Acid phosphatase was utilized as the marker enzyme for lysosomes. The reaction mixture was prepared as follows: 5 mg of naphthol AS-BI phosphoric acid were dissolved in 0.25 ml of dimethylformamide and diluted to 25 ml with distilled water and further diluted to 50 ml with 0.2M sodium acetate buffer (pH 5.2). 30 mg fast blue BB salt was added and the solution was filtered through Whatman No. 1 filter paper. Gels were incubated in the dark for at least 3 hours at 37°C, washed and scanned at 660 nm.

The succinic-INT reductase reaction was utilized to detect the mitochondrial fraction. The reaction mixture contained 50mM sodium succinate, 25mM sucrose, 1 mg/ml p-iodonitrotetrazolium violet (INT) and 50mM potassium phosphate buffer (pH 7.4). Gels were stained in the dark for at least 1 hour at 37°C, washed and scanned at 680 nm.

The peroxidase reaction was utilized to detect peroxisomes and heme proteins. The reaction mixture was prepared by dissolving 25 mg o-dianisidine dihydrochloride in 12.5 ml of methanol, diluting with 25 ml 0.2M sodium acetate buffer (pH 5.2) and, just before use, adding 12.5 ml of 3% hydrogen peroxide. Gels were immersed in 25% methanol for 20 minutes prior to immersion in the reaction mixture for at least 1 hour (in the dark) at ambient temperature. The gels were "destained" in 25% methanol to remove the unreacted o-dianisidine and scanned at 450 nm in distilled water.

Acridine orange, which binds strongly to nucleic acids, was used to detect nuclei. The dye solution contained 2% (w/v) acridine orange, 1% (w/v) lanthanum chloride and 15% (v/v) acetic acid. The gels were immersed in 7.5% (v/v) acetic acid for 10 minutes prior to staining and incubated in the dye solution for 4 hours at ambient temperature. Destaining was accomplished with 7.5% acetic acid and the gels scanned at 490 nm in distilled water.

Following localization of the subcellular fractions, the gels were cut into sections corresponding to the fractions and the Ce content of the sections was determined by gamma scintillation spectrometry. Each of the 7 individual gels obtained via polymerization in the gel mold (procedure A above) of the contents of a single gradient tube had a volume equivalent to 7% of the volume of the gradient excluding the cushion. Therefore, when sections of the individual gels were counted, the total activity associated with a subcellular fraction was calculated from the fraction of the total volume of the gradient actually counted. With procedure B (above) the entire content of each gradient tube was polymerized to form a single gel which was cut into sections and counted. Thus, the total activity associated with any given fraction was obtained directly. The total activity applied to a gradient was determined by counting an aliquot of the sample, identical to the one applied to the gradient, which had been reserved for this purpose at the time the gradient was loaded.

RESULTS

One day post administration, the livers of the Ce exposed animals exhibited signs of fatty infiltration. They were mottled and light in color and, following centrifugation of the homogenates to obtain the nuclear pellet, the supernatants gave evidence of lipid levels considerably higher than found in control liver homogenates.

A representative subcellular distribution of Ce in the liver [obtained utilizing density gradient procedure B (described above)] at 1, 3 and 7 days post administration is presented in Table 14. The ANOVA of the distribution, as a function of time, is presented in Table 15. Two animals in the 1 day group had extraordinarily high percentages of Ce in their nuclear fractions and low percentages in their pellets. (Other than evoking individual variability, there is, at present, no explanation for this finding). Thus, the means and standard deviations for the nuclear and "Pellet" fractions are presented both including and excluding these animals.

The highest percentages of recovered Ce was found in the "Pellet". This fraction is comprised of the materials having a density greater than that of the nuclear fraction. The percentage of Ce in the "Pellet" increased from 45 to 53 from 1 to 7 days post exposure. The nuclear fraction contained the second highest percentage of Ce. However, in contrast to the "Pellet" fraction, the percentage of Ce in this fraction decreased from 28 to 14 from 1 to 7 days post administration. The mitochondria contained the third highest percentage of Ce which remained relatively invarient throughout the period of observation. As in the "Pellet", the percentages of Ce in both the microsomes and the cytosol increased across time, while the peroxisome content decreased to a constant level at 3 days. The differences in Ce content across time were significant for all fractions except the mitochondrial.

V. CONCLUSIONS

1. The Tissue/Organ Distribution and Alterations in Open Field and Exploratory Behavior Following Acute Exposure to Ce

Between 4 hours and 7 days post administration, Ce administered via the i.g. route at a level as high as the LD_{25}, had no significant effect on open field (ambulations and rearings) or "hole-in-board" exploratory behaviors of the adult mouse. Apparently, this was due to the fact that little Ce is absorbed from the gut (Table 3). These data are in agreement with earlier studies in cattle (39), employing radiocerium as a marker for gastrointestinal motility, in which up to 95% of the administered dose was recovered in the feces within 4 days. Despite low uptake, the doses employed were toxic to the mouse. Gastritis and enteritis were demonstrated histologically in these animals. However, it is not known if Ce was directly responsible for such lesions. It is possible that osmotic phenomena may have been involved in view of the high solute concentrations employed.

A different situation prevailed when Ce was administered via the s.c. route. At short times post administration, open field and exploratory behaviors were significantly depressed, systemic distribution of Ce was substantial and behavioral alterations were correlated with tissue/organ Ce levels.

An inverse relationship between behavior and brain and lung Ce levels (Table 11) was found. Of all tissues studied, the brain correlated most strongly with behavior. This is of particular interest in that the same treatment groups which had exhibited depressed open field behavior (which occurred at 4 hr. and 1 day post s.c. administration of Ce at the LD_{25} level and at 4 hr. post administration of the LD_5 dose) also exhibited detectable brain Ce levels. In contrast, the brain Ce levels of all other groups were essentially at or below the detection limit of our instrumentation. Moreover, there are numerous reports in the literature of lanthanum interfering with "calcium sites" on nerve cells (40-42). Cerium, the next element in the lanthanide series, may have a similar effect which, in this study, was expressed as depressed open field and exploratory behavior.

After the brain regions, the strongest behavioral correlation involved the lung (Table 11). The lung accumulated substantial quantities of Ce (Table 3). However, histological examination revealed no abnormalities attributable to Ce. Thus, the relationship between lung Ce levels and behavior remains unclear. Inhalation studies in beagle dogs (33) revealed long term pulmonary retention of approximately 35% of the administered dose of $^{144}CeCl_3$. This is 7 times greater than the quantity retained following ingestion (less than 5%) (39). Taken together, these data suggest that inhalation of Ce may be a considerably greater hazard than ingestion.

It has been observed that Ce induces fatty infiltration of the liver of the rat at levels considerably lower than those employed in our studies (12, 13,36,37). Histological evidence of liver damage and regeneration was found in the animals employed in our study of open field behavior. Thus, it is possible that hepatotoxicity may have been involved, in some way, in the observed behavioral alterations, even though liver Ce levels were only rather weakly correlated with open field behavior (Table 11).

The spleen is of interest in that it contained the highest Ce concentration of any tissue examined (Table 3) and its Ce level was the only one positively correlated with behavior (Table 11). The spleen removes damaged erythrocytes and leucocytes from the circulation and may have acquired its Ce load from such cells. The high Ce concentration in the spleen and the positive correlation with behavior, suggest that sequestration of Ce by the spleen may function to spare more sensitive potential target tissues, such as the brain from perturbation. Indeed, the small negative correlations which were observed between Ce levels in the spleen and brain regions are consistent with such a "protective" function.

It is possible, of course, that the hypoactivity observed in the open field behavioral measures of animals receiving Ce via the s.c. route was mediated by a secondary effect of Ce, namely, that it made the animals physically ill and disinclined to move. However, it should be noted that no behavioral effects were observed in animals receiving Ce via the i.g. route, even though histopathological analysis provided evidence of acute gastritis and enteritis with a duration of at least 7 days post administration.

If the animals were hypoactive simply because they were physically ill, it would be anticipated that the i.g. dosed animals would certainly have been physically ill at least through day 7 post administration and, therefore, hypoactive. The s.c. dosed animals which showed no evidence of gastritis or enteritis exhibited significant open field behavioral alterations which were strongly correlated with brain Ce levels. Furthermore, s.c. dosed animals also exhibited alterations in exploratory behavior (Figure 1). The exploratory task employed is substantially less dependent on the gross activity level of the animals and, therefore, suggests that the effects of Ce were not limited to a simple decrease in the general activity level.

In summary, Ce administered via the s.c. route achieves general systemic distribution and depresses open field and exploratory behavior up to 24 hours post administration, possibly by interaction with the CNS.

High Ce doses administered i.g., while toxic, did not effect either ambulations or rearings in the open field nor exploratory behavior. Probably, this was due to the poor uptake of Ce from the gut which, consequently, limited systemic distribution. Peroral exposure to Ce would appear to present little danger of acute toxicity, since the dose used in our studies were considerably greater than any generally anticipated to occur from environmental exposure.

If Ce compounds are incorporated into fuels, inhalation will constitute another route of environmental exposure. A much higher percentage of Ce

appears to be retained following inhalation than ingestion (39,42). However, even via s.c. administration, the lung accumulates Ce (Table 3) and lung Ce levels are inversely correlated with open field activity (Table 11). These findings suggest that inhalation of Ce may be a considerably greater acute hazard than ingestion and must be investigated before Ce (compounds) can be considered "safe" for widespread environmental dispersion.

2. Activity Wheel Study

The results of this study indicate that a single dose of Ce at the s.c. LD_5 level can significantly depress general activity and that this effect is persistant (Figure 2). This relative depression was maintained, along with a monotonic increase in performance, for all groups (LD_5, LD_{25} and controls) over time. The most depressed scores for the Ce exposed groups occurred during the 24 hour period following Ce administration. It is difficult to interpret this effect since the animals did not exhibit any obvious signs of illness or incapacitation. It appears that a single exposure to Ce, at relatively high levels (LD_5, LD_{25}), has a substantial effect on total (24 hour) activity level. Perhaps total activity, as assessed by the activity wheel, is a more sensitive index of subtle effects induced by Ce exposure than is the short time sample obtained with such measures as the open field.

3. Passive Avoidance

Compared to sodium citrate, acute Ce citrate administration was associated with an increase in latency in both trials of the passive avoidance learning task. This was probably another manifestation of the depressed activity observed in the acute open field and activity wheel studies. Relative to the citrate controls, learning of the passive avoidance task was not adversely effected by Ce administration.

4. Active Avoidance Behavior

Single doses of Ce at the LD_5 and LD_{25} levels had no effect on two-way active avoidance learning by the mouse. However, consistent with the results of the open field, activity wheel and passive avoidance studies (see above), Ce at the s.c. LD_{25} level depressed activity. The depressed activity did not influence the learning of the task.

5. Social Behavior

As in the open field, activity wheel, passive avoidance and active avoidance studies, Ce (at the LD_5 and LD_{25} levels) depressed gross activity. Thus, the control (citrate) animals were significantly more active than either group of Ce exposed animals (which were not significantly different). Although mobility was depressed, Ce had no dramatic effect on the social behaviors that were investigated. However, multivariate ANOVA of the data revealed a dosage by day of observation interaction. This interaction is difficult to assess. Taken at face value, it would appear that Ce, at high dose levels (LD_{25}), does affect a measure of social behavior (distance maintained between pairs of animals), but the effect has a long latency and does

not appear until 3 days post exposure. The absence of more consistent patterns of effects on social behavior indicates that further study is required before confident conclusions can be generated.

6. The Tissue/Organ Distribution and Alterations in Open Field and Exploratory Behavior Following Exposure to Repeated Doses of Ce

Exposure to repetitive s.c. doses of Ce citrate at the LD_1 level resulted in the accumulation of Ce mainly in the spleen, liver and skeleton. No decrease in Ce levels was observed in these tissue up to 98 days after the last dose was administered. Cerium levels in the liver, kidneys, pancreas and testes and possibly in the stomach and duodenum appeared to plateau following the fifth or sixth dose. However, spleen and bone continued to accumulate Ce even after exposure was ended. Blood and brain levels were very low; exhibiting counting rates (^{141}Ce) near background. It is of interest to note that the blood Ce levels remained low throughout the course of the experiment. Similar results were found in our acute s.c. study (see above). Cerium obviously is cleared very efficiently from the blood. Judging from their Ce concentrations across time, the spleen, liver and bone appear to be involved in the clearance mechanism.

Although Ce is rapidly cleared from the blood, it is not rapidly excreted and toxic effects could result from long-term retention unless Ce is sequestered in some non-toxic form.

Exposure to repetitive s.c. doses of Ce significantly depressed ambulations in the open field and marginally depressed "hole-in-board" explorations across all subjects. However, these behavioral measures did not correlate with tissue/organ Ce levels and appear to indicate that the depressive effect of Ce is not a one step process depending directly on Ce levels in some tissue(s). Rather, it probably involves a chain of events culminating in depressed activity. Such a process is not uncommon in biological systems.

In the acute study described previously, highly significant depressions of ambulations, rearings and explorations at 4 hours and 1 day post s.c. administration occurred. The doses employed in that study were much higher (136 and 173 mg Ce/kg) than the individual doses (20 mg Ce/kg) used in the repeated dose study and could account for the differential behavioral results. Detectable Ce levels were found in the brain coincident with depressed behavior in the acute study whereas no detectable brain Ce levels were found in the repeated dose study. It may be that the depression of ambulations and exploratory behavior observed in the repeated dose study was due to small amounts of Ce in the central nervous system. However, the magnitude of this relationship was too small to detect statistically given the sample size and the sensitivity of the Ce assay.

Both the acute and repeated dose studies demonstrated that exposure to Ce can induce behavioral changes in the mouse. Furthermore, the hepatoxicity of Ce is well known (4,7,12,13,31,32). Consideration of these findings along with the long biological residence time of Ce, it seems reasonable to conclude that widespread environmental dispersion of Ce could be potentially

hazardous to man. However, much more information must be accumulated before the risks of environmental Ce dispersion can be accurately assessed.

7. Neonatal and Adult Offspring Studies

Maternal administration of Ce on Day 12 of gestation affected the development of certain offspring behaviors. Conversely, offspring of mothers receiving Ce on Day 7 of gestation did not differ from control offspring in the neonate and adult behavioral measures investigated. Whether the differential effects of day of exposure were due to differences in the intrinsic sensitivity of the developing organism at these stages of gestation or to placental permeability factors were not examined.

The most consistent effect of Ce was on offspring weight. Neonatal weight was reduced in offspring exposed to Ce *in utero* via maternal administration and in the offspring reared by mothers who received Ce on Day 2 *post partum*: during lactation/suckling. The significant prenatal x postnatal interaction indicates that the treatment the foster mother receives can modify the rate of weight gain of the offspring she rears. Thus, exposure of pregnant females to a single dose of Ce at the s.c. LD_1 level had a relatively long-lived effect on the mother that was reflected postnatally in a decreased rate of offspring growth. This effect may be direct, through residual Ce in the milk of mothers treated during gestation or indirect through effects on maternal behavior manifested, for example, as neglect of offspring and reflected in such factors as ineffective suckling or lack of grooming as reported by Young (43).

The latter possibility appears reasonable since studies in our laboratory revealed that Ce is not transmitted to offspring via the milk of mothers exposed during gestation to the dose levels of Ce employed in our experiment. However the reduced weights observed in offspring exposed to Ce prenatally may indicate, for example, that the developing fetuses received inadequate nutrition because of covert maternal intoxication resulting in impaired transport of nutrients across the placenta, Ce induced metabolic dysfunction in the offspring that resulted in impaired ability to utilize nutrients, the production of milk of inferior nutritional quality or, perhaps, all, or combinations, of these possibilities.

8. Maternal Behavior

The offspring retrieval data indicate that pups exposed to Ce *in utero* are retrieved in preference to control pups. Unfortunately, activity was not tested on day 3 *post partum*, the day retrieval was tested. Conceivably, Ce pups were preferentially retrieved because they were less active and of lighter weight. This supports the finding of Cone (44) that less active, lighter pups are retrieved in preference to active and heavier pups.

9. Liver Subcellular Distribution of Ce

The highest percentages of Ce were found in the pellet (about 50%) and the nuclei (28% decreasing to 14%) (Table 14). About half of the Ce found in the nuclei of cells examined 1 day post exposure appeared to have moved

into the pellet, microsomal and cytosolic fractions of cells by 3 to 7 days post exposure. While no data are available on the mechanism of this transfer, Ce may be transported out of the nuclei bound to mRNA (30) produced in conjunction with regenerative processes in the liver (45). Cerium may exist in the pellet in the form of insoluble phosphates and hydroxides (46-48). Insoluble forms of Ce also may be trapped within organelles or bound to macromolecules (proteins, RNA or DNA) that are normally associated with them.

Based on the ultrastructural and biochemical studies of Salas et al. (32) and our subcellular distribution study, it is possible to devise a mechanism to account for the rapid appearance and disappearance of Ce induced fatty infiltration of the liver. Thus, in the liver, Ce forms insoluble compounds with hydroxide ions (47), various biologically important phosphate compounds including nucleotide mono-, di-, and triphosphates (44,45) and various macromolecules, including DNA, RNA, and proteins. By direct interaction with ATP, Ce would reduce cellular levels of high energy phosphate. In addition, Ce appears to interfere in some way, with the availability of adenine for the biosynthesis of ATP. Salas et al. (32) have found that pretreatment with ATP, or adenine or tryptophan, which are ATP precursors, prevented the Ce induced fatty infiltration of the liver. As the levels of ATP fall (32), energy-dependent processes, including protein and RNA synthesis, would become inhibited. In addition, transcription might be inhibited by the direct binding of Ce to DNA.

Low liver ATP levels would trigger fatty infiltration of the liver through normal metabolic mechanisms. Since liver glycogen and blood glucose levels would be depleted, peripheral lipid stores would have to be mobilized in order to raise liver ATP levels. In that lipids are secreted by the liver in the form of lipoproteins, Ce inhibition of protein synthesis would contribute to high liver lipid levels by reducing lipoprotein biosynthesis.

As the availability of "active" Ce became reduced (via precipitation or binding to macromolecules, etc.), ATP and other phosphate-containing molecules could again begin to accumulate and the liver would begin to return toward the normal state (regeneration).

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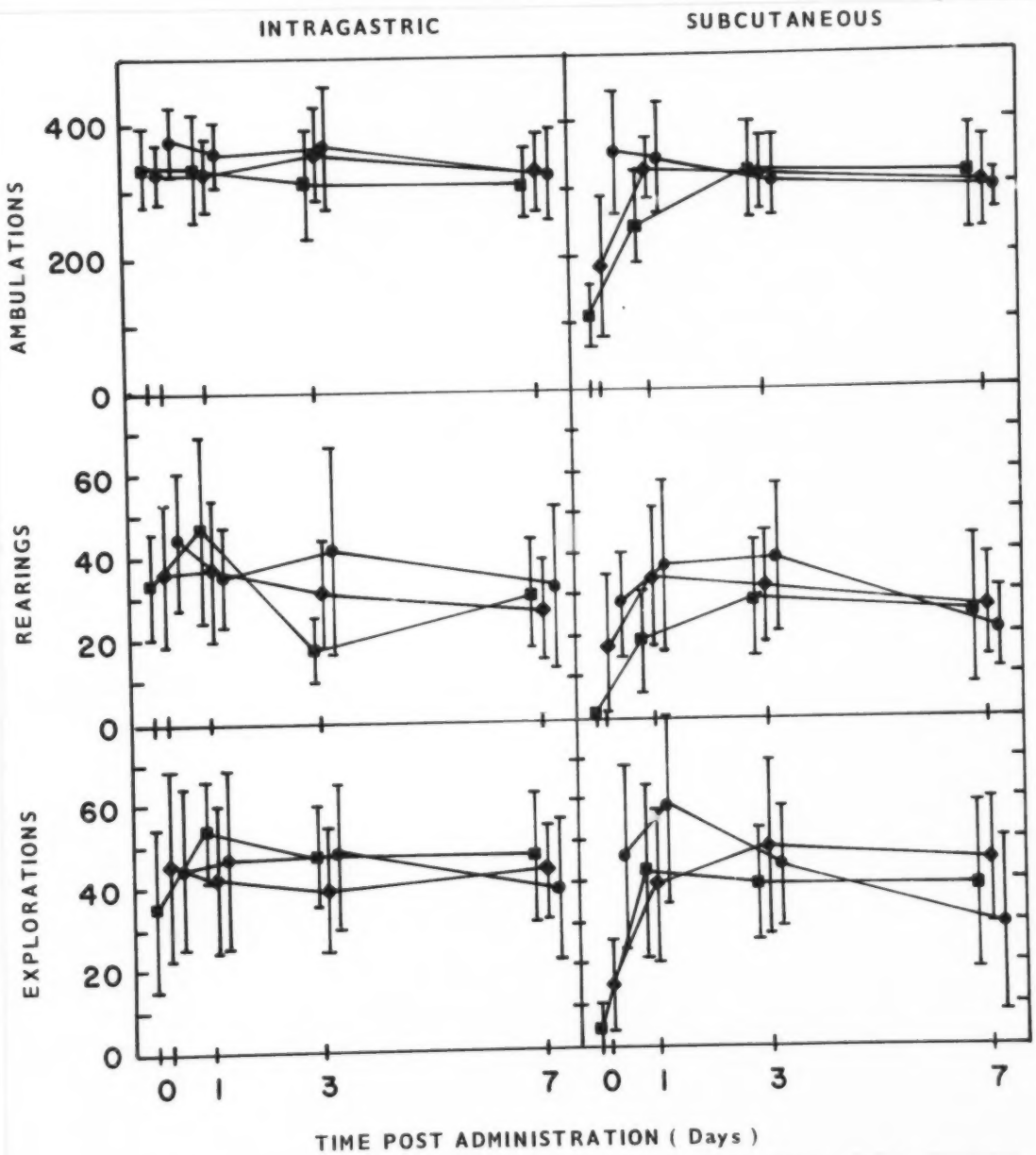


Figure 1. Effects of Cerium (citrate) on open field and "hole-in-board" exploratory behavior. Subjects (adult male mice) were observed (automated open field or automated exploratory apparatus) at 4 hrs., 1, 3 or 7 days post i.g. or s.c. administration of either $CeCl_3$ (complexed 1:3 with sodium citrate) or sodium citrate solution (controls). Total ambulations, rearings and explorations (means $\pm$ s.d.) for 5 minute observation periods are shown. Treatments were: (■) Ce at the 7 day LD_{25} level (i.g. = 1163 ppm Ce; s.c. = 173 ppm Ce). (◆) Ce at the 7 day LD_5 level (i.g. = 1000 ppm Ce; s.c. = 136 ppm Ce). (●) Sodium citrate (i.g. = 6140 ppm sodium citrate, s.c. = 917 ppm sodium citrate).

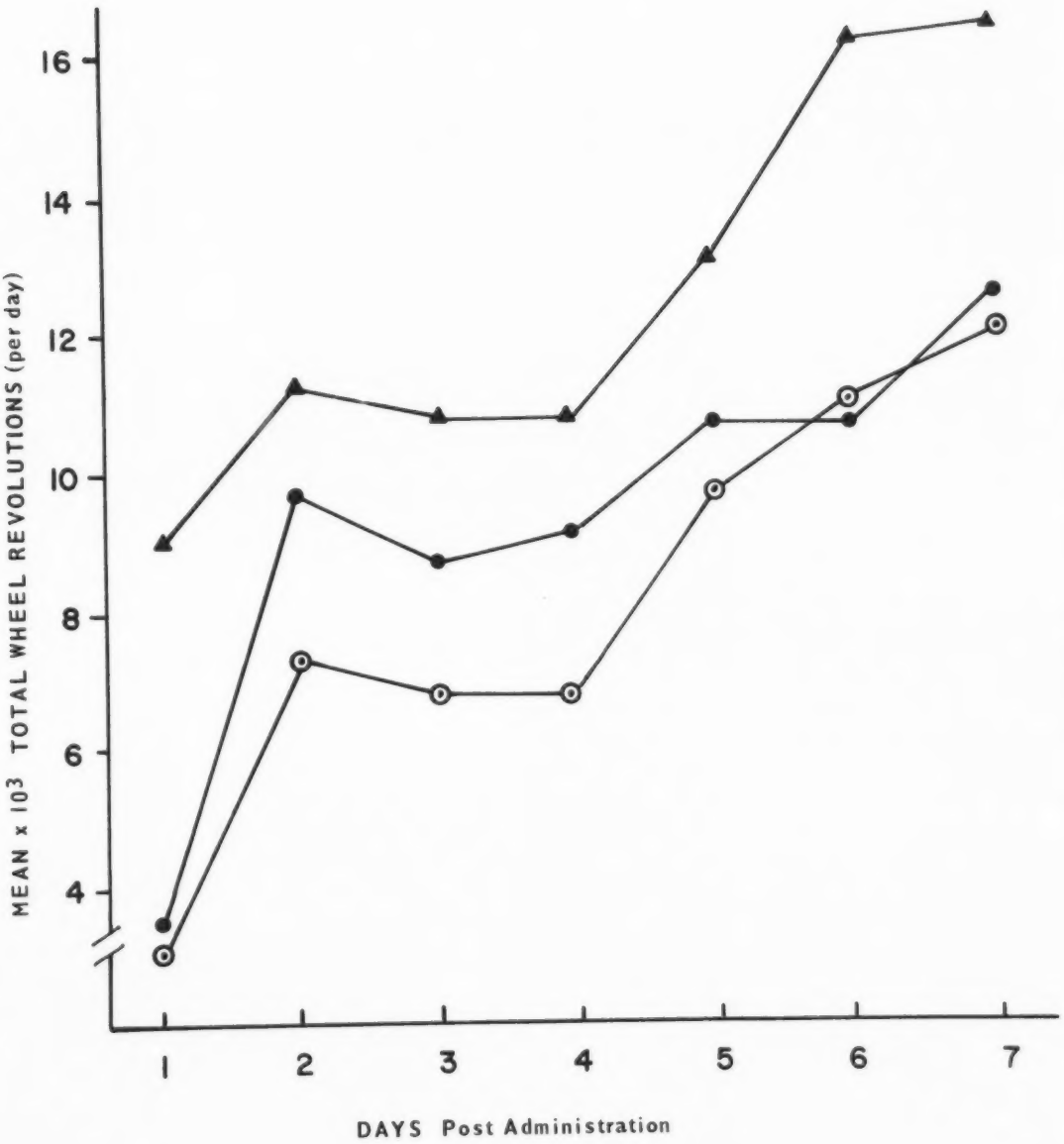


Figure 2. Effects of Cerium (citrate) on wheel running performance. One hour before light onset on Day 0, subjects (adult male mice) received either a single s.c. dose of Ce at the LD_5 or LD_{25} level or sodium citrate solution (controls). Total wheel revolutions were recorded continuously during the dark phase of the light cycle (lights on at 7:00 p.m., off at 7:00 a.m.). Symbols: ▲ sodium citrate; ● Ce at the 7 day LD_{25} level; ⊙ Ce at the LD_5 level.

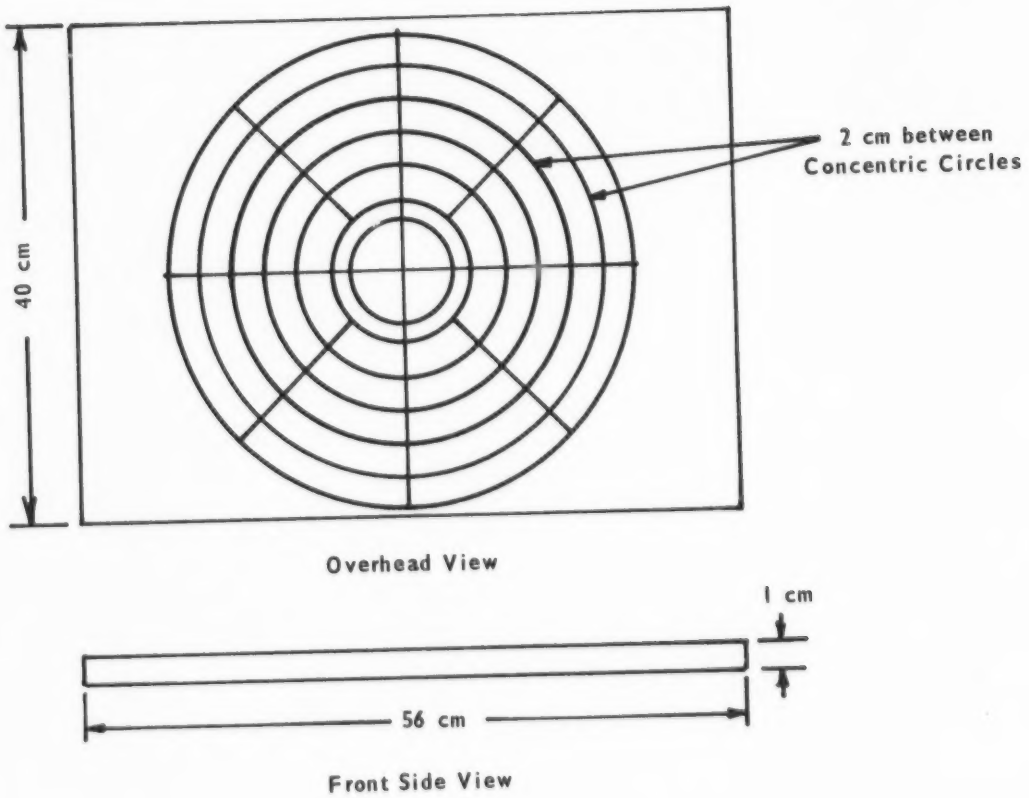
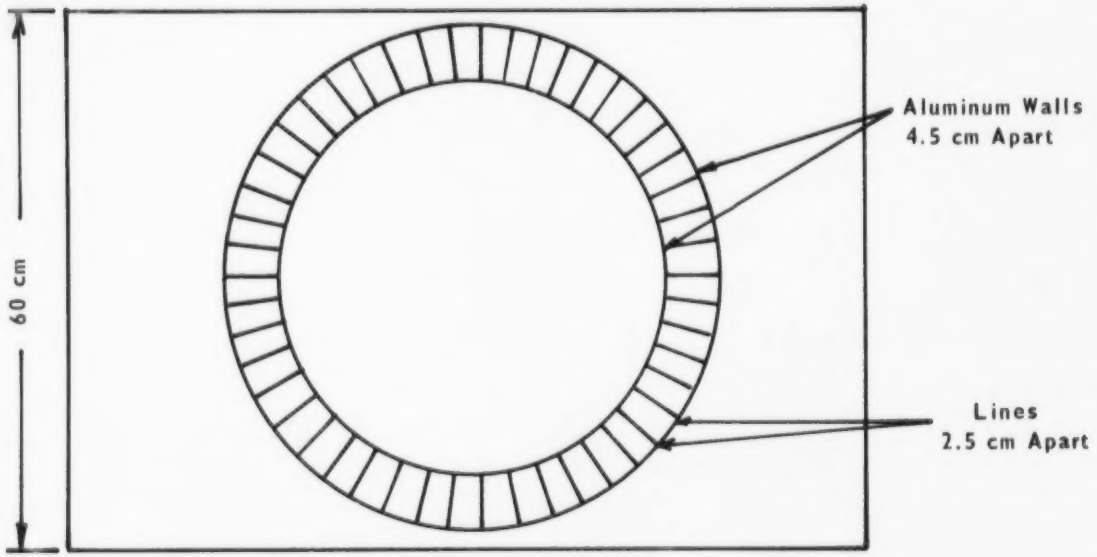
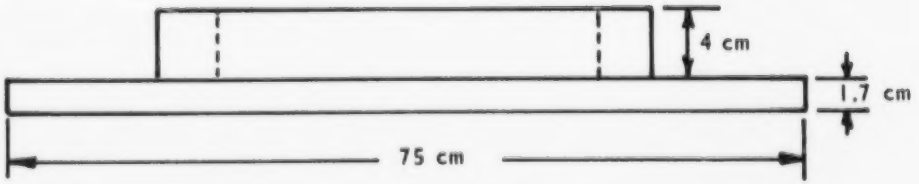


Figure 3. Activity board. The field was painted a light, flat grey. The circles and radii were dark grey. The center circle had a diameter of 5.5 cm.



Overhead View.



Front Side View

Figure 4. Circular runway. The runway was enclosed by concentric aluminum walls. The floor of the runway was painted a light, flat grey and the lines, dark grey.

TABLE 1. EFFECT OF INCREASING Ce DOSE[a] ON 7 DAY VIABILITY OF GRAVID[b] AND NONGRAVID ANIMALS

Dose level	7 day viability	
	Nongravid	Gravid
68	----	10/10
86	----	10/10
104	----	9/10
122	10/10[c]	8/10
140	10/10	8/10
157	8/10	5/10
175	7/10	5/10
192	4/10	3/10
210	6/10	1/10
228	6/10	1/10
246	2/10	0/10
264	1/10	0/10
282	1/10	----
300	0/10	----
318	0/10	----

[a] The Ce dose administered was based on maternal weight at time of injection.

[b] All mice were injected on day 12 of gestation.

[c] Numerator = number surviving; denominator = total N.

TABLE 2. Ce LETHAL DOSE LEVELS AS DETERMINED BY LINEAR REGRESSION ANALYSIS OF 7 DAY VIABILITY

Lethality[a]	Nongravid Dose[b]	Nongravid Range[c]	Gravid[d] Dose	Gravid[d] Range
LD_1	117	71.6-142	80.2	43.6-103
LD_5	138	95.8-161	94.4	55.8-119
LD_{11}	151	112-172	108	73.1-129
LD_{25}	174	143-193	131	100-153
LD_{40}	193	168-212	150	127-172
LD_{50}	205	183-226	166	147-189
LD_{60}	218	198-244	179	160-204
LD_{75}	241	220-283	199	181-237
LD_{90}	280	249-360	226	198-280
LD_{95}	305	266-419	254	218-361
LD_{99}	360	300-559	301	251-497

[a] Lethality levels are expressed as the percent mortality expected within 7 days after Ce administration.

[b] Ce dose, in ppm administered on the basis of maternal weight, calculated to produce the corresponding lethality.

[c] The $p<0.05$ probability range of the corresponding dose.

[d] All mice were injected on day 12 of gestation.

TABLE 3. TISSUE/ORGAN Ce CONCENTRATION IN μg Ce/g (WET WEIGHT) TISSUE (PPM) - ACUTE EXPOSURE

	Intragastric route							
	4 Hour				1 Day			
	1163 PPM Ce		1000 PPM Ce		1163 PPM Ce		1000 PPM Ce	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	2.1	1.5	2.3	4.2	0.2	0.2	0.1	0.2
Liver	16.7	19.5	14.3	26.0	9.8	4.8	8.8	7.5
Spleen	60.5	72.8	13.5	32.5	6.0	2.7	5.5	5.2
Kidney	7.1	8.5	6.2	13.1	0.4	0.5	0.5	0.6
Lung	16.5	18.2	4.7	7.3	5.3	15.2	28.8	90.0
Stomach	2977.8	945.2	2358.5	1010.2	1818.4	1198.3	1722.2	1150.1
Duodenum	1140.0	918.9	581.3	392.9	418.8	575.5	296.2	215.3
Muscle	9.0	8.3	2.4	3.4	1.0	1.1	0.3	0.9
Pelt	0.4	0.8	0.3	0.3	0.1	0.2	0.3	0.4
Cerebrum	0.6	0.7	0.5	0.6	0.1	0.3	0.0	0.3
Cerebellum	0.5	0.8	0.0	0.6	0.2	1.5	0.6	1.0
Brain Stem	1.0	2.9	0.9	2.3	0.1	0.6	0.2	1.0
	3 Days				7 Days			
	1163 PPM Ce		1000 PPM Ce		1163 PPM Ce		1000 PPM Ce	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	0.1	0.2	0.0	0.2	0.1	0.1	0.1	0.2
Liver	9.4	6.0	9.5	4.5	10.1	4.8	9.6	5.9
Spleen	4.9	4.1	6.0	3.8	8.7	7.2	1.3	8.7
Kidney	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2
Lung	1.1	2.5	3.5	9.2	9.2	23.3	11.6	29.0
Stomach	1138.3	623.1	921.0	320.1	814.2	258.0	736.5	254.6
Duodenum	204.7	102.8	217.6	112.7	257.9	150.7	240.6	105.8
Muscle	0.1	0.5	0.0	0.5	0.0	0.6	0.1	0.5
Pelt	0.0	0.4	0.1	0.3	0.1	0.5	0.2	0.6
Cerebrum	0.1	0.3	0.0	0.3	0.0	0.2	0.2	0.3
Cerebellum	0.0	1.4	0.0	1.1	0.5	0.7	0.6	0.9
Brain Stem	0.0	0.6	0.0	0.5	0.2	1.1	0.2	0.5

TABLE 3. (continued)

	Subcutaneous route							
	4 Hours				1 Day			
	173 PPM Ce		136 PPM Ce		173 PPM Ce		136 PPM Ce	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	2.5	1.2	8.2	13.1	1.1	0.7	1.0	0.4
Liver	555.2	209.0	438.5	104.5	557.1	204.7	441.2	108.7
Spleen	995.0	354.7	1398.4	732.6	1901.7	731.4	2151.9	614.0
Kidney	61.8	24.6	33.5	26.5	48.9	37.1	33.2	27.0
Lung	1389.6	703.8	159.4	219.0	811.0	778.9	341.7	368.9
Stomach	32.3	76.5	68.4	133.4	4.4	3.1	4.6	2.6
Duodenum	5.3	2.1	55.5	113.5	3.2	3.4	1.8	0.7
Muscle	3.5	4.3	1.1	0.8	2.2	3.8	1.3	0.5
Pelt	75.2	113.5	157.9	145.3	86.6	127.9	33.4	52.8
Cerebrum	1.2	0.5	0.7	0.7	0.5	0.5	0.2	0.2
Cerebellum	2.9	3.9	1.4	1.8	0.5	0.8	0.3	0.4
Brain Stem	1.4	0.7	0.9	1.0	0.5	0.6	0.3	0.2
	3 Days				7 Days			
	173 PPM Ce		136 PPM Ce		173 PPM Ce		136 PPM Ce	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.1
Liver	534.4	124.9	364.1	83.1	474.6	120.3	412.3	83.9
Spleen	2065.6	648.1	1650.5	717.3	1016.8	298.7	870.6	393.2
Kidney	39.4	14.3	19.2	9.5	39.4	15.4	22.5	17.4
Lung	310.7	232.6	95.3	46.9	447.0	303.6	225.6	198.0
Stomach	6.6	4.5	2.6	1.5	3.9	1.2	2.4	1.4
Duodenum	2.9	1.3	1.8	0.8	2.7	1.5	1.8	1.7
Muscle	1.1	0.5	1.1	0.5	1.2	0.6	0.8	0.4
Pelt	5.7	10.3	9.8	13.8	2.3	2.9	1.7	2.8
Cerebrum	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2
Cerebellum	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.3
Brain Stem	0.2	0.2	0.2	0.1	0.3	0.4	0.3	0.2

TABLE 4. ANALYSES OF VARIANCE OF TISSUE/ORGAN CONCENTRATIONS OF CERIUM - ACUTE EXPOSURE[a]

Variable	Effects Being Tested						
	Time	Route	Dose Within IG	Dose Within SC	Time x Route	Time x Dose Within IG	Time x Dose Within SC
Multivariate d.f.	36/393.691	12/133	12/61	12/61	36/393.691	36/180.959	36/180.959
F-ratio	6.90[b]	131.53[b]	1.32	4.68[b]	5.64[b]	1.15	1.99[c]
Univariate d.f.	3/144	1/44	1/72	1/72	3/144	3/72	3/72
F-ratio[e]							
Blood	11.08[b]	3.55	-	1.61	1.51	-	1.84
Liver	1.03	886.86[b]	-	14.22[b]	0.85	-	0.51
Spleen	15.18[b]	516.46[b]	-	0.03	16.05[b]	-	2.02
Kidney	4.69[b]	178.61[b]	-	15.51[b]	1.43	-	0.03
Lung	7.16[b]	92.36[b]	-	30.97[b]	6.74[b]	-	6.23[b]
Stomach	22.81[b]	287.38[b]	-	0.40	20.73[b]	-	0.61
Duodenum	10.93[b]	74.48[b]	-	1.70	9.10[b]	-	2.05
Muscle	15.29[b]	0.03	-	4.22[d]	6.40[b]	-	1.50
Pelt	8.60[b]	25.75[b]	-	0.20	8.52[b]	-	2.3
Cerebrum	28.08[b]	22.55[b]	-	7.21[c]	8.52[b]	-	1.67
Cerebellum	6.90[b]	7.31[c]	-	1.98	5.48[b]	-	0.90
Brain Stem	7.47	1.62	-	4.16[d]	0.09	-	1.24

[a] The data were analyzed by a 3-way analysis of variance. The factors were:
Time after administration (4 hr; 1, 3, and 7 days)
Route of administration (i.g., s.c.)
Dose, nested within route (i.g. - 1163, 1000 mg Ce/kg body weight (bw);
s.c. - 173, 136 mg Ce/kg bw).

[b] $p < 0.001$

[c] $p < 0.01$

[d] $p < 0.05$

[e] Univariate F-ratios were not examined if the corresponding multivariate F-ratio was not significant.

TABLE 5. TISSUE/ORGAN Ce CONCENTRATIONS IN μg Ce/g (WET WEIGHT) TISSUE (PPM) - REPEATED EXPOSURE

Cumulative dose (mg Ce/kg) ppm[a]	Time (days) after first exposure	N (subjects per cell)	Blood[b] Mean	Blood SD	Liver Mean	Liver SD	Kidney Mean	Kidney SD	Spleen Mean	Spleen SD	Pancreas Mean	Pancreas SD	Lung Mean	Lung SD
20	3	10	0.04	0.03	28.80	7.14	1.09	0.38	27.34	10.25	0.19	0.09	2.59	0.45
40	6	10	0.03	0.03	47.53	3.32	3.18	5.17	38.21	15.36	0.24	0.07	3.71	1.08
60	9	10	0.04	0.04	73.36	16.72	3.23	1.17	83.65	43.43	0.59	0.56	11.32	12.46
80	12	10	0.05	0.06	81.93	16.50	3.63	0.90	94.54	44.76	0.60	0.21	9.07	2.86
100	15	10	0.06	0.05	111.02	19.76	5.08	1.28	133.74	33.61	0.94	0.78	9.07	1.12
120	18	8	0.04	0.02	130.59	29.46	6.23	1.13	175.98	64.62	0.86	0.27	12.42	3.58
140	21	7	0.03	0.03	118.38	11.60	5.50	0.95	160.55	35.99	0.82	0.42	13.19	1.65
160	24	9	0.04	0.03	147.31	24.97	6.18	2.63	195.25	46.02	1.10	0.48	14.67	2.85
180	27	7	0.03	0.03	151.61	19.24	6.23	2.57	227.88	96.71	0.98	0.18	15.95	5.10
200	30	7	0.04	0.02	155.27	37.32	6.55	2.15	228.50	118.61	1.08	0.37	16.27	5.01
200	69	9	0.09	0.23	157.06	57.51	7.56	3.10	315.83	217.35	1.16	1.47	14.24	4.83
200	125	6	0.18	0.16	152.78	44.73	6.01	1.57	341.32	124.05	1.84	1.20	13.49	2.38

[a] Each dose (s.c.) contained 20 mg Ce/kg body weight as the $CeCl_3$: sodium citrate, 1:3 complex, pH 7.4. Animals were sacrificed three days after their last injection except for the last two groups which were sacrificed 42 and 98 days after their last injection.

[b] Concentrations near detection limit and should be regarded as upper limits rather than accurate values.

(continued)

TABLE 5. (continued)

Cumulative dose (mg Ce/kg) ppm a	Time (days) after first exposure	N (subjects per cell)	Stomach		Duodenum		Testes		Skeletal muscle	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	3	10	0.68	2.27	0.78	0.37	0.37	0.12	0.11	0.07
40	6	10	0.87	0.37	0.96	0.41	0.54	0.24	32.04	44.46
60	9	10	1.67	0.61	2.11	0.75	0.82	0.17	24.53	26.53
80	12	10	2.21	0.55	2.40	1.22	1.14	0.75	23.12	28.25
100	15	10	2.92	1.35	3.59	1.98	1.38	0.35	89.54	114.56
120	18	8	3.29	1.28	4.31	1.86	1.72	0.46	32.62	26.89
140	21	7	2.99	1.56	2.87	0.43	1.51	0.35	35.91	56.17
160	24	9	4.05	1.46	3.80	1.18	1.84	0.25	47.71	42.37
180	27	7	3.69	1.60	4.49	1.82	1.83	0.33	30.18	29.18
200	30	7	4.65	2.27	3.59	1.53	1.84	0.54	26.90	17.90
200	69	9	4.51	2.05	3.77	3.93	1.85	0.68	37.80	44.57
200	125	6	4.03	2.91	2.63	1.11	2.09	0.67	54.25	50.94

(continued)

TABLE 5. (continued)

Cumulative dose (mg Ce/kg) ppm[a]	Time (days) after first exposure	N (subjects per cell)	Bone		Cerebrum[b]		Cerebellum[b]		Brain Stem[b]	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	3	10	3.71	1.14	0.09	0.05	0.2	0.2	0.09	0.07
40	6	10	6.69	5.41	0.05	0.05	0.2	0.2	0.06	0.06
60	9	10	12.70	3.74	0.13	0.11	0.3	0.3	0.08	0.08
80	12	10	16.48	10.69	0.07	0.03	0.2	0.2	0.10	0.08
100	15	10	22.02	12.98	0.08	0.03	0.2	0.2	0.12	0.07
120	18	8	26.62	11.70	0.16	0.08	0.3	0.3	0.16	0.10
140	21	7	15.74	5.82	0.11	0.06	0.3	0.3	0.14	0.08
160	24	9	21.02	9.81	0.13	0.06	0.2	0.1	0.12	0.10
180	27	7	34.01	29.18	0.13	0.05	0.3	0.2	0.16	0.07
200	30	7	27.60	13.38	0.12	0.06	0.3	0.2	0.13	0.12
200	69	9	30.91	9.04	0.13	0.11	0.2	0.3	0.19	0.25
200	125	6	39.17	9.01	0.77	0.65	1.3	1.2	1.13	0.47

TABLE 6. DAM AND EMBRYO/FETAL TISSUE Ce DISTRIBUTION FOLLOWING DAY 7 ADMINISTRATION (PPM)

	Day post administration						
	1	2	3	5	20	25	34
	Day of gestation				Days post partum		
	8	9	10	12	7	12	21
Blood[a]	0.38±14.00	0.26± 0.02	0.29± 0.04	0.03± 0.01	0.02± 0.01	0.02± 0.01	0.01± 0.00
Liver[a]	320.00±43.00	333.00±23.00	242.00±16.00	195.00±19.00	133.00±21.00	152.00±14.00	150.00±19.00
Kidney[a]	11.50± 2.00	12.30± 1.50	8.83± 0.47	7.46± 0.32	6.29± 0.68	5.93± 1.63	4.72± 0.51
Spleen[a]	209.00±36.00	296.00±39.00	272.00±85.00	205.00±19.00	93.10±31.60	117.00±18.00	267.00±41.00
Muscle[a]	0.93± 0.06	2.61± 1.22	1.34± 0.39	0.45± 0.15	0.63± 0.14	0.41± 0.11	0.71± 0.26
Uterus[a]	3.59± 0.90	2.40± 0.16	2.97± 0.81	1.34± 0.25	18.40± 2.20	6.03± 1.46	2.82± 0.74
Cerebrum[a]	0.12± 0.01	0.14± 0.02	0.13± 0.03	0.06± 0.04	0.04± 0.02	0.04± 0.02	0.06± 0.01
Cerebellum[a]	0.17± 0.04	0.24± 0.09	0.17± 0.08	0.14± 0.04	0.04± 0.02	0.06± 0.01	0.06± 0.01
Lung[a]	28.30± 3.20	16.60± 2.40	27.70± 7.40	11.50± 2.90	4.73± 0.89	5.46± 0.36	6.08± 0.78
Embryo/ Fetus[b]	5.71± 1.22	3.38± 0.91	3.08± 0.43	0.82± 0.20	----------	----------	----------

[a] Mean ± SEM for 5 animals.

[b] Mean ± SEM for all embryos/fetuses from 5 mothers.

TABLE 7. DAM, PLACENTAL AND FETAL TISSUE Ce DISTRIBUTION FOLLOWING DAY 12 ADMINISTRATION

	Days post administration						
	1	2	3	5	15	20	29
	Day of gestation				Days post partum		
	13	14	15	17	7	12	21
Blood[a]	0.57± 0.31	0.28± 0.10	0.31± 0.06	0.05± 0.02	0.03± 0.02	0.05± 0.02	0.01± 0.01
Liver[a]	184.00±18.00	152.00± 8.00	144.00±36.00	177.00± 14.00	140.00±12.00	187.00±26.00	178.00±21.00
Kidney[a]	8.78± 2.02	7.83± 0.98	6.72± 0.94	6.59± 0.88	15.60± 4.10	8.24± 0.97	9.46± 1.81
Spleen[a]	98.00±52.30	130.30±10.30	196.00±45.00	340.00±120.00	167.00±28.00	324.00±60.00	389.00±48.00
Muscle[a]	0.65± 0.13	0.53± 0.11	0.51± 0.02	0.42± 0.04	0.53± 0.16	0.62± 0.07	1.55± 0.40
Uterus[a]	4.45± 0.04	4.18± 1.17	2.93± 0.51	1.48± 0.42	17.50± 3.80	35.60±11.30	79.90±18.30
Cerebrum[a]	0.11± 0.02	0.07± 0.02	0.06± 0.03	0.05± 0.01	0.05± 0.01	0.11± 0.04	0.09± 0.03
Cerebellum[a]	0.14± 0.06	0.11± 0.05	0.12± 0.05	0.11± 0.04	0.18± 0.05	0.12± 0.03	0.09± 0.03
Lung[a]	17.60± 8.40	13.30± 0.90	12.90± 2.60	12.10± 1.80	6.18± 1.33	8.97± 1.62	6.00± 1.89
Fetus[b]	0.04± 0.01	0.02± 0.01	0.05± 0.01	0.02± 0.01	----------	----------	----------
Placenta[b]	3.73± 0.13	3.83± 0.91	2.51± 0.43	1.62± 0.39	----------	----------	----------

[a] Mean ± SEM for 5 animals.

[b] Mean ± SEM for all embryos/fetuses from 5 mothers.

TABLE 8. DAM AND OFFSPRING TISSUE Ce DISTRIBUTION FOLLOWING DAY 2 POST PARTUM ADMINISTRATION

	Days post partum				
	3	4	5	12	21
Blood[a]	0.47± 0.15	0.70± 0.01	0.03± 0.01	0.03± 0.01	0.13± 0.07
Liver[a]	207.00± 5.0	237.00±15.00	174.00±12.00	182.00±17.00	187.00±24.00
Kidney[a]	15.40± 2.90	11.80± 2.40	9.80± 0.50	10.30± 0.90	8.83± 2.07
Spleen[a]	492.00±19.00	308.00±68.00	214.00±55.00	125.00±16.00	235.00±67.00
Muscle[a]	1.37± 0.36	1.96± 0.06	0.75± 0.31	0.75± 0.24	1.33± 0.26
Uterus[a]	87.00±28.00	88.20± 3.50	108.00±27.00	92.30±49.00	101.00±64.00
Cerebrum[a]	0.11± 0.01	0.21± 0.06	0.09± 0.05	0.07± 0.02	0.40± 0.18
Cerebellum[a]	0.21± 0.04	0.21± 0.07	0.13± 0.04	0.10± 0.01	0.40± 0.25
Ovary[a]	21.20± 0.40	28.20± 3.80	----------	18.50± 5.90	9.27± 1.92
Bone[a]	13.70± 1.40	18.90± 5.60	18.40± 1.70	11.00± 1.20	15.10± 3.00
Lung[a]	19.70± 2.60	25.90± 4.50	8.40± 0.20	7.07± 0.69	6.41± 0.26
Offspring[a]	0.31± 0.10	0.43± 0.16	0.12± 0.02	----------	----------

[a] Mean ± SEM for 5 animals.

TABLE 9. ANALYSIS OF VARIANCE OF OPEN FIELD BEHAVIORAL MEASURES - ACUTE EXPOSURE[a]

	Variable effects being tested						
	Time	Route	Dose	Time x route	Time x dose	Route x dose	Time x route x dose
Multivariate d.f.	6/430	2/215	4/430	6/430	12/430	4/430	12/430
F-ratio	4.51[b]	18.24[b]	6.96[b]	6.37[b]	3.17[b]	1.51	2.77[b]
Univariate d.f.	3/216	1/216	2/216	3/216	6/216	2/216	6/216
F-ratio[e]							
Ambulations	7.00[b]	30.85[b]	12.18[b]	9.70[b]	4.89[b]	-----	4.12[b]
Rearings	3.84[c]	17.78[b]	6.83[b]	6.76[b]	2.04	-----	2.29[d]

[a] The data were analyzed by a 3-way analysis of variance. The factors were: time after administration (4 hr.; 1, 3 and 7 days) route of administration (i.g., s.c.) and dose (LD_{25}, LD_5 or citrate only).

b = $p < 0.001$
c = $p < 0.01$
d = $p < 0.05$
e = Univariate F-ratios were not examined if the corresponding multivariate F-ratio was not significant.

TABLE 10. ANALYSIS OF VARIANCE OF BEHAVIORAL MEASURES - ACUTE EXPOSURE VIA THE S.C. ROUTE[a]

	Variable effects being tested		
	Time	Dose	Time x dose
Multivariate d.f.	6/214	4/214	12/214
F-ratio	7.94[b]	6.48[b]	4.02[b]
Univariate d.f.	3/108	2/108	6/108
F-ratio			
Ambulations	14.66[b]	11.30[b]	7.81[b]
Rearings	8.36[b]	7.13[b]	2.11

[a] The data were analyzed by a 2-way analysis of variance. The factors were:
time after administration (4 hr.; 1, 3 and 7 days)
dose (LD_{25}, LD_5 or citrate only).

[b] = $p < 0.001$

TABLE 11. CORRELATIONS BETWEEN TISSUE/ORGAN Ce LEVELS AND AMBULATIONS AND REARINGS - ACUTE EXPOSURE

	Complete design		S.C. route only	
Test of hypothesis of no association	Overall F = 4.78, d.f. = 24/292, p <0.001		F = 3.04, d.f. = 24/132, p <0.001	
	Ambulations	Rearings	Ambulations	Rearings
	F = 8.14 d.f. = 12/147 p <0.001	F = 4.14 d.f. = 12/147 p <0.001	F = 6.82 d.f. = 12/67 p <0.001	F = 2.88 d.f. = 12/67 p <0.003
Correlation coefficients	d.f. = 158		d.f. = 78	
Blood	-0.325[a]	-0.153	-0.382[a]	-0.231
Liver	-0.386[a]	-0.270[b]	-0.236[b]	-0.097
Spleen	-0.161	-0.145	+0.253[b]	+0.192
Kidney	-0.433[a]	-0.276[a]	-0.359[a]	-0.212
Lung	-0.488[a]	-0.388[a]	-0.461[a]	-0.346[a]
Stomach	+0.186	+0.261[a]	-0.372[a]	-0.277[b]
Intestine	+0.110	+0.075	-0.354[a]	-0.230
Muscle	-0.082	+0.011	-0.268[b]	-0.221
Pelt	-0.221[b]	-0.189	-0.145	-0.153
Cerebrum	-0.422[a]	-0.343[a]	-0.615[a]	-0.517[a]
Cerebellum	-0.384[a]	-0.194	-0.496[a]	-0.309[a]
Brain Stem	-0.289[a]	-0.125	-0.665[a]	-0.490[a]

a = p <0.01

b = p <0.05

TABLE 12. CELL MEANS AND STANDARD DEVIATIONS FOR WHEEL REVOLUTIONS RECORDED FOUR HOURS AFTER Ce OR CITRATE ADMINISTRATION - ACUTE EXPOSURE

Treatment	Means	Standard Deviation
LD_5	216.7	255.3
LD_{25}	440.7	339.7
Citrate	3275.5	1178.7

TABLE 13. EFFECTS OF Ce ON MOUSE OPEN FIELD AND EXPLORATORY BEHAVIOR - REPEATED EXPOSURE[a]

Analysis of variance of behavioral measures			
	Effects being tested		
Variable	Number of exposures	Treatment	Exposures X treatment
Multivariate			
d.f.	27/485.45	3/166	27/285.45
F-ratio[b]	1.85 ($p < 0.01$)	2.36 (N.S., $p < 0.07$)	1.01 (N.S.)
Univariate			
d.f.	9/168	1/168	9/168
F-ratio[b]			
Ambulations	1.19 (N.S.)	5.22 ($p < 0.05$)	----------
Rearings	3.00 ($p < 0.01$)	1.12 (N.S.)	----------
Explorations	2.09 ($p < 0.05$)	3.14 (N.S., $p < 0.08$)	----------

[a] The data were analyzed by a 2-way analysis of variance. The factors were: number of exposures and treatment (Ce citrate or sodium citrate).

[b] Univariate F-ratios normally are not examined if the corresponding multivariate F-ratio is not significant at the $p < 0.05$ level. However, in these studies, while formally retaining the critical level of $p < 0.05$, we are noting significance levels up to $p < 0.10$.

TABLE 14. LIVER SUBCELLULAR Ce DISTRIBUTION AT 1, 3 AND 7 DAYS POST ADMINISTRATION (PERCENT OF Ce RECOVERED)

	Time post administration (days)					
	1		3		7	
Fraction	Mean	SD	Mean	SD	Mean	SD
Cytosol	2.34	0.39	4.40	0.73	7.17	0.53
Microsomes	7.32	1.29	12.49	0.88	13.92	1.52
Mitochondria	10.33	1.80	10.09	2.21	8.28	1.30
Peroxisomes	6.37	1.40	3.64	0.73	3.18	0.73
Nuclei	36.70 (28.16)[a]	14.31 (4.22)[a]	17.49	4.95	14.14	2.01
"Pellet"[b]	37.47 (45.73)[a]	13.32 (4.05)[a]	51.88	2.89	53.29	1.60
% of Ce Recovered	96.34	1.73	90.64	2.82	88.49	3.55

[a] Values obtained omitting 2 animals with extraordinarily high nuclei and low pellet values.

[b] This term denotes all materials with a density greater than that of the nuclear fraction.

TABLE 15. ANALYSIS OF VARIANCE OF LIVER SUBCELLULAR Ce DISTRIBUTION

Variable	Time Effect
Multivariate d.f.	12/20
F-ratio	13.67[a]
Univariate d.f.	2/15
F-ratios	
Cytosol	118.72[a]
Microsomes	45.66[a]
Mitochondria	2.01
Peroxisomes	17.75[a]
Nuclei	11.08[a]
	(16.26)[a]
Pellet	7.23[b]
	(9.22)[bd]
% Ce Recovered[c]	11.22[a]

[a] $P < 0.001$

[b] $P < 0.01$

[c] Not included in the multivariate F-ratio.

[d] Values obtained omitting 2 animals in the 1 day group with extraordinarily high nuclei and low pellet values.